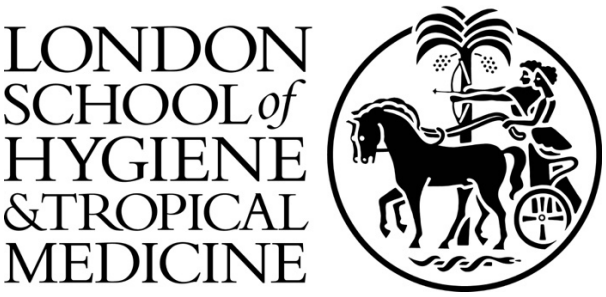

Determining Effectiveness of New Approaches to Dengue Vector Control in Cambodia

John Hustedt

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Department of Infectious Disease Epidemiology
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London School of Hygiene and Tropical Medicine

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LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

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Student ID Number	**Lsh1406618**	**Title**	**Mr**
First Name(s)	**John Christian**		
Surname/Family Name	**Hustedt**		
Programme of Study	**PhD**		
LSHTM Email (if this is no longer active, please provide an alternative)	**john.hustedt@lshtm.ac.uk**		

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Abstract

Background: Dengue is an important public health problem with an estimated 390 million infections annually worldwide, and an estimated 1.6 million infections annually in Cambodia. Due to the rise in dengue cases, and the current lack of widely available effective vaccines and therapeutics there is an urgent need to come up with more effective, sustainable, and locally appropriate vector control methods.

Methods: A cluster randomized trial with three arms was designed to assess the impact of guppy fish (*Poecilia reticulata*), in combination with the larvicide pyriproxyfen (PPF), and Communication for Behavioural Impact (COMBI) activities, on entomological indices over one year in Cambodia. In addition, entomology data was used to determine the ability of the Premise Condition Index (PCI) to predict *Aedes* mosquito density and prioritize vector control interventions.

Results: The guppy only intervention arm was able to decrease the number of *Aedes* females (Density Ratio (DR)=0.49) and Pupae Per Person (DR=0.56) by roughly half compared to the control arm. There were no statistical differences identified between the two intervention arms. All other entomological indices showed similar statistically significant reductions in intervention arms compared to the control arm. Data from the KAP and qualitative assessments showed community acceptance of interventions. Despite statistically significant associations between PCI scores and adult and immature *Aedes* densities, receiver operating characteristic curves suggest the PCI was a poor predictor of whether premises had higher densities of immature and adult *Aedes* mosquitoes.

Conclusions: The effectiveness of interventions demonstrated in the trial along with community acceptance suggests guppies should be considered as vector control tools in Cambodia as long as the benefits outweigh any potential environmental concerns. The PCI results suggest caution is warranted in programmatic use of PCI in areas of similar geography and mosquito abundance.

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Acronyms and abbreviations

ADB	Asian Development Bank
AUC	Area Under the Curve
BI	Breteau Index
Bti	*Bacillus thuringiensis israelensis*
CHW	Community Health Worker
CI	Confidence Interval
COMBI	Communication for Behavioural Impact
FGD	Focus Group Discussions
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit
HC	Health Centre
HH	Household
HI	House Index
IDI	In-depth Interviews
IE	Inhibition of Adult Emergence
IVM	Integrated Vector Management
KAP	Knowledge, Attitudes, and Practice
LN	Long-Lasting Insecticidal Netting
MC	Malaria Consortium
NAMRU-2	US Naval Medical Research Unit-2
NDCP	National Dengue Control Program
OD	Operational District
PCI	Premise Condition Index
PPF	Pyriproxyfen
PPH	Pupae Per House
PPP	Pupae Per Person
ROC	Receiver Operating Characteristic
RR	Rate Ratio
TSC	Technical Steering Committee
WHO	World Health Organization

Chapter 1: Introduction

Dengue is transmitted through bites of infected *Aedes* mosquitoes, principally *Aedes aegypti* [1]. Although dengue virus infection in humans is clinically apparent in only approximately 25% of cases, it can lead to wide range of clinical manifestations from mild fever to potentially fatal shock syndrome [1]. Despite current research devoted to drug discovery and supportive treatments there is currently no effective antiviral cure for dengue and therefore treatment remains supportive [2]. Dengue infections are caused by four closely related viruses named DEN-1, DEN-2, DEN-3, and DEN-4. Research suggests lifelong immunity is developed after infection but is type-specific [3]. The occurrence of severe symptoms is frequently associated with a secondary infection of a different serotype [1].

Approximately 3.9 billion people in 128 countries are at risk of dengue infection [4]. The disease affects most of the world's tropical and sub-tropical regions and has become the most rapidly spreading mosquito-borne viral disease [1,5]. There were an estimated 390 million infections in 2010, of which 96 million were clinically apparent [4]. These estimates are based on data from various sources including published literature, surveillance data, news reports, and consultations with experts [5]. As the data themselves are of varying quality and completeness the estimates have large confidence intervals. However, the estimates do represent a global consensus of experts that suggests the number of infections is increasing over time and expanding geographically [4]. Between 2010 and 2020 the World health Organization (WHO) is aiming to reduce morbidity and mortality from dengue by least 50% and 25%, respectively [6].

In 2015, the first commercial dengue vaccine, Dengvaxia® (Sanofi-Pasteur), came on the market. However, the vaccine had less than ideal overall efficacy (67%) and extremely low efficacy against serotype DENV-2 (35%) [7,8]. Additionally, safety concerns led the WHO to recommend only offering the vaccine to those who (1) are aged 9-45 years, and (2) live in areas with high seroprevalence (>80%) or who are seropositive [9]. Due to the limitations of the vaccine, the WHO recommended that vaccination should be "part of an integrated dengue prevention and control strategy together with well executed and sustained vector control" [9]. Academics and leading dengue control experts have said that regardless of the efficacy of future vaccines, there is growing consensus that no single intervention will be sufficient to control dengue disease [10]. Vector control therefore remains a key part of any dengue control program, and the integration of locally accepted and effective methods is needed [11].

Determining which vector methods are best for any locality can be complicated as there are many options and insecticide resistance is growing in many locations [12]. WHO separates vector control methods into three groups: biological controls, chemical controls, and environmental management [13]. Interventions within each group can target immature or adult mosquitoes. The most common chemical controls include larvicides such as temephos, adulticides used in indoor residual spraying and space sprays (deployed by backpack portables, trucks, and aircraft), and personal protection adulticides such as DEET or Picaridin [13]. Biological controls have been the focus of many new research and control projects focused on container treatment (e.g. larvivorous fish, copepods, dragonfly larvae) [13]. Environmental management most often focuses on improvement of water supply and water storage systems, mosquito-proofing of water containers, solid waste management, street cleansing, water

container management, and improving building structures. A recent expert working group convened by the Partnership for Dengue Control reviewed all the evidence available on vector control tools and recommended the following tools for sustained management of dengue vectors: 1) the use of Indoor spraying (preferably with residual insecticides) and perifocal spraying with residual insecticides for adult control, 2) comprehensive container larvicide treatment and container removal, and 3) social mobilization campaigns, environmental management [10].

In addition to the currently commercially available tools, there are several promising technologies focusing on genetic engineering of mosquitoes. The first category of these is the release of genetically modified male mosquitoes which carry a dominant lethal gene, such as the *Aedes aegypti* strains developed by Oxitec [14,15]. However, questions remain of feasibility due to logistic challenges and concerns over costs [16]. In addition, even once the intervention was successful there remains the possibility of mosquito populations returning from nearby areas, necessitating the need for releases indefinitely or until mosquitoes could be eradicated throughout entire continents [17]. Another category is the release of mosquitoes infected with the intracellular bacterium *Wolbachia,* which can establish itself in mosquito populations and suppress arbovirus replication in mosquitoes [18]. The advantage of this method is that continuous releases are not required when the local frequency of Wolbachia in wild *Aedes aegypti* mosquitoes surpasses an unstable equilibrium point, and no potential ecological harms would come from eradicating the mosquito. Recent trials of this technique in Australia concluded that the deployment of *Wolbachia* into *Aedes aegypti* populations can be readily scaled quickly and cost effectively and appears to be effective at stopping local dengue transmission [19]. These

techniques hold great promise, however more evidence is needed to confirm if the results are generalizable and scale up will take time and require large monetary investments.

Multi-sectorial approaches and community involvement are important as the failure of dengue vector control strategies has often been associated with the absence or lack of active community involvement [20]. Integrated Vector Management (IVM) is a rational decision making process used to optimize, and improve the efficacy and cost-effectiveness of vector control resources [11]. IVM strategies guide control programs to move away from single-intervention approaches and promote multi-sectoral approaches to human health. The IVM approach also encourages community engagement and stakeholder involvement in designing and implementing dengue control strategies. Communities take the lead in the project design, planning and decision making which helps to create community acceptance, ownership and ensure sustained community participation in the dengue program [21,22].

In addition to IVM strategies to optimize tools and program design, the Communication for Behaviour Impact (COMBI) toolkit can help develop risk communication, development communication strategies, and health promotion/education materials [23]. The COMBI strategy provides a social mobilization and communication approach that connects knowledge and behaviour, addresses the cost and value of engaging in healthy behaviours, recognizes the gradual stages of behaviour change, and creates a supportive environment for behaviour change [24]. Culturally appropriate, well-informed and multipronged behaviour change communication approaches increase awareness and address the misperceptions surrounding dengue infection and control tools in the communities [25–27]. Utilizing IVM and COMBI

strategies and integrating communities into the selection of tools, program design, and communication strategies and materials can help improve the effectiveness of dengue control programs.

1.1 Dengue situation in Cambodia

Asia records 70% of the global disease burden due to dengue [4] and Cambodia has one of the highest per-capita incidence rates in the region [28]. First identified in Cambodia in 1963 [29], dengue is now considered endemic. A total of 194,726 cases were reported to the National Dengue Control Program (NDCP) between 1980 and 2008 [30]. Between 2003 and 2008, dengue incidence ranged between 0.7 and 3.0 per 1,000 person years [31]. The numbers of suspected cases reported to WHO in 2018 was 9,885 and in the first 23 weeks of 2019 was 2,490 [32,33]. Additionally, 21 deaths were reported during the first 20 weeks of 2019 [34].

However, data reported by NDCP come from a maximum of five hospitalized patients per week in each of the seven sentinel provincial hospitals, and few sentinel health centres, and the National Paediatric Hospital [35]. The restriction on number of patients included is due to funding constraints. Therefore, the surveillance data are useful in monitoring which serotypes are circulating and identifying seasonal patterns, but it is not meant to be a true estimate of dengue burden. The real number of cases has estimated by comparing cohort data to the NDCP surveillance number and estimated to be between 3.9 and 29.0 times higher than those reported by NDCP [36,37]. The most recent modelling data based off published literature, surveillance data, news reports, and consultations with experts estimated an alarming 1.6 million cases or 119 times greater than those reported to WHO by NDCP [4].

Even with the underreported number of cases, the annual cost to society was estimated to be between $3.3 - $14.4 million between 2006-2008 [38]. Since most of this cost falls onto the family, it resulted in 67% of households falling into debt to pay for medical bills [39]. A more recent study showed the average cost of illness per patient in 2016 was $134, and the average monthly household income was $245 USD [40]. Therefore, the cost to households is not negligible and there is a strong need to identify control methods.

1.2 Dengue vector control in Cambodia

Dengvaxia® is licensed in Cambodia, but is only available in a small number of private clinics who choose to purchase it through the producer [41]. Due to the lack of therapeutics or a widely available vaccine, the majority of individuals rely on vector control as the primary means of dengue prevention. Larval surveys have shown that large concrete water jars, drums, and tanks contained over 91% of *Aedes aegypti* larvae in Cambodia [42]. Therefore, much of the focus has been on interventions that could target these household water storage containers.

Since the early 1990s, the primary means of vector control by the Cambodian NDCP has been the use of the organophosphorous larvicide temephos (under the trade name Abate ®) applied in large water storage containers [43]. However, increasing resistance to temephos has been found in Cambodia [12,43,44] and in other parts of Southeast Asia over the last 20 years [6,45–47]. In addition to larviciding regularly, the NDCP uses thermal fogging with pyrethroids (most commonly permethrin and deltamethrin) during outbreak responses. Recent experiments showed all adult *Aedes aegypti* populations tested in Cambodia were highly resistant to permethrin and

seven out of eight showed resistance to deltamethrin [12]. The resistance data show that using temephos for larval control may even be counterproductive possibly fixing resistance in the *Aedes* population, and very few effective adulticides are available for use in general dengue control programs or outbreak response. As suggested by researchers in Cambodia it is imperative that "we must quickly find an alternative" [12].

1.3 Alternative vector control methods

Several alterative vector-control methods have been studied in Cambodia including chemical and biological substances. One of the first was a *Mesocyclops* (type of copepod or group of small crustaceans) project in Kratie province from 2002 to 2004 [48]. Initial results showed a reduction in the *Aedes* population in the intervention area, but by the end of the project larval densities in the intervention area had increased by 62% from baseline. In addition, *Mesocyclops* from the local water sources had various parasites and colonizing them parasite-free required special training that was difficult for the villagers. Another limitation was that many participants did not accept *Mesocyclops* to the same extent as other interventions that were provided by the NDCP such as temephos [49]. Therefore, the NDCP did not attempt any further projects using *Mescoclops*.

In 2004, an evaluation of *Bacillus thuringiensis israelensis* (*Bti*), a Gram-positive, soil-dwelling bacterium showed positive results with significant reductions in the number of pupae for at least 2 and 2.5 months in containers with river and well-water, respectively [18]. Further evaluation from 2005-2011 showed pupal suppression of 91% for eight weeks, and authors claimed a reduction in adult mosquitoes and dengue cases compared to control villages [50]. There were

two separate studies included in one manuscript with the entomology indicators being evaluated in 2005 and 2006 in two communes (one treated and one untreated), and the number of dengue cases recorded in 2010 and 2011 in 11 communes (six treated and five untreated). However, two large unreported limitations exist. The first being that in the initial study the two communes were literally across the street from each other, and therefore it is difficult to tell if the difference was due to the intervention or other differences between the communes. The second is that in the later study the communes were not randomized and those which had a higher number of dengue cases in 2010 were selected for treatment in 2011. Therefore, it is likely that villages with more cases one year will have greater immunity and less cases the following year. In fact, the reduction in cases within the treatment arm (in 2011) was greater in the pre (60%) versus post (53%) *Bti* treatment phase. Additionally, the data used for dengue cases were the numbers reported to NDCP, which have their own limitations discussed earlier. Regardless, there is data from several years to show that *Bti* did have statistically significant decreases in immature mosquitoes and could be considered for future control efforts.

In 2006, jar covers with long-lasting insecticidal netting treated with deltamethrin were found to have significantly fewer pupae per house, and a three-fold decline in *Aedes aegypti* adult females per house [42]. However, the magnitude of the reduction diminished over time due to a gradual reduction of insecticidal effect of the jar covers and a residual deltamethrin life of 22 weeks [42]. Another reason for low effectiveness may have been children not always keeping the jar covering on after extracting water, and using the covers as toys around the house [49] as Khun et al. noted in Cambodia before [25]. Improvements in engineering and design to prevent entry and

egress of mosquitoes, especially when the container is used, and an increase the insecticidal effectiveness may be needed for jar covers to be cost-effective public health interventions [42].

The use of larvivorous fish (*Poecilia reticulata*) was evaluated in 14 Cambodian villages in 2006-2007 [51], and subsequently in a larger study of 28 Cambodian villages in 2009-2011 [52]. Results from the initial study found guppies in 56% of eligible containers, and a 79% reduction in *Aedes* infestation compared to the control [51]. These results led a larger scale-up in 2009–2011. Results showed 88% guppy fish coverage in eligible water containers and a Container Index (CI) and the number of indoor resting adult females near zero at the end of the project (while the control had a CI of 30%) [52]. Container Index is the percentage of water-holding containers infested with larvae or pupae. However, there were additional miscellaneous breeding sites including containers too small for guppy survival. Therefore, additional tools beyond larvivorous fish are required to target these smaller miscellaneous, hard-to-reach and cryptic breeding containers or sites.

Poecilia reticulata, commonly known as the guppy, is one of the most widely distributed tropical fish found on every continent except Antarctica and has even made it to space aboard the USSR biosatellite Cosmos [53]. Guppies were first described in 1859 in Venezuela, and their natural range appears to be Trinidad, Venezuela, Guyana, and Surinam and probably Tobago. Their first documented introduction was from Hawaii to the Philippines in 1905 for mosquito control, however little is known about introductions before that time. They are poeciliids, a group of fish characterized by internal fertilization, viviparity, and the male intromittent organ, the gonopodium [53]. Females are larger than males when an average size of 3-6 cm compared to

1.5-3 cm. All wild male guppies have different colour patterns which is important in attracting females, while females are mostly grey in body colour (Figure 1.1). Females can store sperm in the folds of their ovaries and gonoducts and can continue to fertilize ova for up to eight months. The number of offspring can vary from one to a hundred or more, and at birth guppies are independent and no further parental care is needed [53].

Figure 1. 1: Photo of guppies (source: ResearchGate)

An alternative that can reach all containers is pyriproxyfen (PPF). PPF is a juvenile hormone analogue that interferes with the metamorphosis of juvenile *Aedes* mosquitoes, preventing their development into adults [54]. The results of the first study in 2003 were so promising – at higher doses, inhibition of adult emergence (IE) greater than 87% for 6 months – that a larger second

study was designed [55]. This showed that a novel 5% controlled-release formation led to IE above 90% for 20 weeks, and above 80% for 34 weeks [56]. A slow-release PPF matrix release formulation (Sumilarv® 2MR) has since been developed and is also suitable for containers uninhabitable by guppy fish. The added benefit of Sumilarv® 2MR is that it only requires one distribution every 6 months (the entirety of the rainy season) and cuts down on operational costs as compared to temephos or *Bti* which have a residual efficacy of 2–3 months [57,58]. However, one limitation is that as PPF inhibits immature mosquitoes from become adults, and confusion has been reported over effectiveness due to the presence of live larvae in the water containers (Shafique et al., submitted manuscript).

1.4 Identifying key premises

The identification of key premises is crucial to inform vector control operations – an activity which can be conducted through pupal/demographic surveys of household water containers. However, the ubiquity of water containers tends to make pupal/demographic surveys laborious [59]. Therefore, additional methods have been explored to identify key premises without needing to do extensive pupal/demographic surveys, or enter premises, because owners refusing access to premises has been reported as a key challenge [60]. The Premise Condition Index (PCI) is one such approach that could help prioritize outbreak response in terms of *Aedes* infestation risk [61]. This index evaluates the shade, house, and yard conditions of premises to produce risk strata. In addition to targeting treatment of key premises, this method could potentially be used to prioritize villages or other geographical areas when funding or human resources are insufficient to treat all outbreak areas. Results have varied by geography and mosquito life stage, but if shown to be useful in predicting premises or geographic areas with

greater *Aedes* densities PCI could potentially be used to prioritize interventions when funds are insufficient to treat all areas/houses.

1.5 Overall aims and objectives

The overall aim of the PhD was to determine which new vector control approaches (beyond larviciding with temephos or using pyrethroid sprays) would be most effective in Cambodia. This involved speaking with key stakeholders and designing a trial that could properly evaluate new alternatives. Negotiations with NDCP and other stakeholders in 2014 concluded that, based on past research reported above, the use of larvivorous fish, PPF, and *Bti* held the most promise for the country. Due to limited funds available in Cambodia for dengue control, the use of larvivorous fish (*Poecilia reticulata*) sourced from the original government established colony was recommended for larger water containers (>50 litres) as it was effective, cheap, and easily available. However, as approximately 10% of *Aedes* larvae were in small or miscellaneous containers that are not easily targeted with fish, the use of new controlled release version of PPF (Sumilarv® 2MR) in smaller containers (<50 litres) was recommended and those in which guppies could not survive. The use of Sumilarv® 2MR was suggested over *Bti* or other formulations of PPF as it is long-lasting thereby obviating much of the operational costs involved in larviciding in Cambodia. Integrating IVM and COMBI strategies into any potential project was also recommended.

Although there is evidence suggesting that the use of guppy fish can be beneficial in dengue vector control, a recent review showed that there has never been a cluster randomized trial to evaluate their effectiveness to reduce mosquito indices [62]. Therefore, the aim included

designing a trial which could have the potential to inform the strategic application of community-based distribution of larvivorous fish and PPF in an outbreak, during inter-epidemic periods or for broad-scale application. The specific objectives of the PhD study are outlined below:

1. Perform a systematic literature review on the impact of PPF on *Aedes* mosquitoes including to (1) Determine the effect of PPF on a range of endpoints including percentage inhibition of emergence, larval mortality, and resistance ratios; and (2) Determine the different uses, strengths, and limitations of PPF in vector control of *Aedes*.
2. Design a cluster randomized trial in which villages were randomized to either (1) three interventions (guppies, Sumilarv® 2MR, and COMBI activities), (2) two interventions (guppies and COMBI activities), or (3) control (standard vector control).
3. Carry out the results of the trial and report on all outcomes mentioned in the protocol.
4. Determine the ability of the PCI to predict premises with adult and immature *Aedes* mosquitoes in Cambodia.

1.6 Thesis outline

The thesis is presented in a research paper style submitted in accordance with London School of Hygiene and Tropical Medicine regulations. The prepared and published articles are included without adaptation and there is therefore some repetition between chapters on the study context. This has been minimized where possible. Where research papers included supplementary material for publication these have been included either at the end of the relevant chapter, or in the Appendix section. The thesis contains six chapters, which include one published paper and three prepared (unpublished) manuscripts. A brief overview of each chapter follows:

- Chapter 1 is an introduction to the global and country specific dengue situations, vector control tools, and methods for prioritizing vector control activities. This section also includes thesis aims and objectives of the PhD.

- Chapter 2 is a systematic literature review on the impact of PPF on *Aedes* mosquitoes. The results of the review will be used to provide evidence to control programs on the suitability of PPF as a vector control tool. This study helped in determining recommendations for Cambodia following the results of the main cluster randomized trial discussed in Chapter 3 and 4 and can inform control strategies in other interested jurisdictions across the world.

- Chapter 3 is the protocol for the main cluster randomized trial presented in this thesis. This protocol was registered in the ISRCTN registry and published in the journal *Trials*.

- Chapter 4 reports the results of the trial described in Chapter 3. The interventions included in the trial are listed above in Section 1.5. It also includes some results reported in Chapter 2 to further strengthen the recommendations around PPF use in Cambodia.

- Chapter 5 reports the results of the association between the PCI and presence of immature and adult *Aedes aegypti*. It also evaluates the ability of the PCI to accurately predict households with higher *Aedes* densities. This work utilizes entomology and PCI data collected in the main trial reported in Chapter 4. The kind of results that are to be

produced could potentially identify strategies for prioritization of vector control activities where resources are scarce.

- Chapter 6 provides further discussion and future directions relating to each of the results chapters. In addition, the chapter provides an overall summary which brings in evidence from other fields such as ecology and discusses public and stakeholder engagement in the material contained in the thesis.

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Chapter 2: Use of pyriproxyfen in control of *Aedes* mosquitoes: a systematic literature review

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

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First Name(s)	John Christian		
Surname/Family Name	Hustedt		
Thesis Title	Determining Effectiveness of New Approaches to Dengue Vector Control in Cambodia		
Primary Supervisor	john.hustedt@lshtm.ac.uk		

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Stage of publication	**Not yet submitted**

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I led the conception and methodological development of the review, wrote the first draft and submitted the protocol to the International Prospective Register of Systematic Reviews. Subsequently, I completed the first database searches and data extraction and wrote up the first draft of the manuscript.

SECTION E

Student Signature	John Hustedt
Date	July 21, 2019

Supervisor Signature	[redacted]
Date	25 July 2019

Use of pyriproxyfen in control of *Aedes* mosquitoes: a systematic review

John Hustedt[1,3*], Ross Boyce[2], John Bradley[1], Jeffrey Hii[3], Neal Alexander[1]

[1] MRC Tropical Epidemiology Group, London School of Hygiene and Tropical Medicine (Keppel Street, London, WC1E 7HT, United Kingdom)

[2] Division of Infectious Diseases, University of North Carolina at Chapel Hill, (130 Mason Farm Road, Chapel Hill, NC 27599)

[3] Malaria Consortium (House # 91, St. 95, Boeung Trabek, Chamkar Morn; PO Box 2116, Phnom Penh 12305, Cambodia)

*corresponding author

Email: johnhustedt@gmail.com

Systematic Review Registration Number: PROSPERO 2016 CRD42016046772

Key Words: Pyriproxyfen, Vector Control, *Aedes*, Systematic Review

Abstract

Background: Dengue is the most rapidly spreading arboviral disease in the world. The current lack of fully protective vaccines and clinical therapeutics creates an urgent need to identify more effective means of controlling *Aedes* mosquitos, the principal vector of dengue. Pyriproxyfen (PPF) is an increasingly used hormone analogue that prevents juvenile *Aedes* mosquitoes from becoming adults and being in capable of transmitting dengue. The objectives of the review are to (1) Determine the effect of PPF on endpoints including percentage inhibition of emergence, larval mortality, and resistance ratios; and (2) Determine the different uses, strengths, and limitations of PPF in control of *Aedes.*

Methodology/Principle Findings: A systematic search was applied to PubMed, EMBASE, Web of Science, LILACS, Global Health, and the Cochrane database of Systematic Reviews. Out of 1,369 records, 91 studies met the inclusion criteria. Nearly all fit in one of the following four categories 1) Efficacy of granules, 2) Auto-dissemination/horizontal transfer, 3) use of ultra-low volume thermal fogging (ULV), thermal fogging (TF), or fumigant technologies, and 4) assessing mosquito resistance. PPF granules have consistently efficacious results of 90-100% inhibition of emergence for up to 90 days. The evidence is less robust but promising regarding PPF dust for auto-dissemination and the use of PPF in ULV, TF and fumigants. PPF has a very favourable mammalian toxicity profile, and its safety is the product are well established in the literature. Several studies also found that while mosquito populations were still susceptible to PPF, the lethal concentrations increased among temephos-resistant mosquitoes compared to reference strains.

Conclusions/Significance: The evidence is strong that PPF does increase immature mortality and adult inhibition in settings represented in the included studies, however future research should focus on areas where there is less evidence (e.g. auto-dissemination, sprays) and new use cases for PPF. A better understanding of the biological mechanisms of cross-resistance between PPF, temephos, and other insecticides will allow control programs to make better informed decisions.

Author summary

Many important diseases are spread by *Aedes* mosquitoes including dengue, chikungunya, Zika, and yellow fever. Dengue cases are increasing worldwide and there is a lack of effective vaccines and therapeutics. Additionally, mosquitoes have become resistant to commonly used insecticides. Pyriproxyfen (PPF) is an insecticide that prevents juvenile *Aedes* mosquitoes from becoming adults. The objective of this review was to determine the effect of PFF on immature and adult mosquitoes and determine different use cases, strengths, and limitations. A systematic search was applied to scholarly databases where 91 full text articles met the inclusion criteria. Nearly all included studies fit in four categories, 1) granules, 2) auto-dissemination, 3) ultra-low volume spray, thermal fogging, and fumigant formulations, and 4) mosquito resistance. While mosquito populations were still susceptible to PPF, the concentrations needed to kill a majority of mosquitoes increased among those resistant to temephos (a commonly used insecticide). The evidence is strong that PPF granules do increase immature mortality and adult inhibition, however evidence for other forms and uses is still weak or could be increased. Better understanding of the cross-resistance between PPF, temephos, and other insecticides will allow control teams to make better informed decisions.

2.1 Introduction

Dengue is the most rapidly spreading mosquito-borne viral disease in the world, with a 30-fold increase in incidence over the past 50 years and an expansion into new geographic areas [1]. Dengue infection is caused by bites of infected *Aedes* mosquitoes, principally *Aedes aegypti*. Dengue has a wide clinical spectrum that ranges from asymptomatic infection to severe disease that manifests with vascular leakage and end-organ failure and is associated with a high rate of morbidity and mortality [1]. With an estimated 3.6 billion people in 124 countries at risk of contracting the disease [2] and 390 million dengue infections occurring each year (of which 96 million develop clinical symptoms) [3] the dengue virus has become a leading cause of illness in the tropics and subtropics [4].

Academics and leading dengue control experts have expressed that regardless of the efficacy of future vaccines, there is growing consensus that no single intervention will be sufficient to control dengue disease [5]. Vector control therefore remains a key part of any dengue control program, and the integration of locally accepted and effective methods is needed [6].These methods together with the development of new vaccines [7], genetic control of mosquitoes [8,9], and new therapeutic drugs [10] will be essential in reducing dengue incidence. One insecticide that has been increasingly used is pyriproxyfen (PPF). PPF is a hormone analogue that interferes with the metamorphosis of juvenile *Aedes* mosquitoes, preventing their development into adults capable of transmitting the dengue virus [11]. A recent systematic review assessing the community effectiveness of PPF found it is highly effective in controlling the immature stages of dengue transmitting mosquitoes, and to a smaller degree adult mosquito population, however they excluded field studies without a control and any efficacy studies performed under laboratory conditions [12]. In this review we are extending the breadth of the systematic review by

identifying all evidence (including lab and semi-field studies and use in combined or novel products) on the effect of PPF on *Aedes* mosquitoes.

Objectives:

The objectives of the review were to (1) Determine the effect of PPF on survivorship of *Aedes* life stages; and (2) Determine the different uses, strengths, and limitations of PPF in vector control of *Aedes.*

2.2 Methods

Search strategy and eligibility criteria

This review follows the guidelines as laid out in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [13] (Appendix 2.1). It was carried out between July 2016 and October 2016, with an update in March-April 2019. All data were extracted by two independent researchers, and discrepancies were resolved by consensus. All studies reporting on the use of pyriproxyfen in control of *Aedes or Stegomyia* as a single agent or combined with other control measures were eligible for inclusion.

Data sources and search strategy

Studies were identified by searching electronic databases, scanning reference lists of articles and consultation with experts in the field. No limits were applied for language in case there was an available English translation. If no translation was available only English and Spanish articles were evaluated. The search was applied to PubMed, EMBASE, Web of Science, LILACS, Global Health, and the Cochrane Database of Systematic Reviews. The International Commission on Zoological Nomenclature (ICZN) which governs the nomenclature aspects of

zoological taxonomy changed the name of the genus *Aedes* to *Stegomyia* [14]. However, here we follow the suggestion of the *American Journal of Tropical Medicine and Hygiene*, made in consultation with several other journals [15], to continue to use *Aedes* as the genus name. Nevertheless, we have also searched based on *Stegomyia.* The search terms in Table 2.1 were applied to all databases.

Table 2. 1: Search Terms Used for Systematic Review

Pyriproxyfen AND Mosquito Control [MESH]
Pyriproxyfen AND Insect Control [MESH]
Pyriproxyfen AND Insect Vectors [MESH]
Pyriproxyfen AND Disease Vectors [MESH]
Pyriproxyfen AND Communicable Disease Control [MESH]
Pyriproxyfen AND Dengue
Pyriproxyfen AND *Aedes*
Pyriproxyfen AND *Stegomyia*

Study selection

For each search, titles and abstracts were imported into Endnote (Thompson Reuters, Philadelphia, PA, USA), duplicates were removed, and the remaining records were screened. Full texts of potentially relevant records were retrieved and assessed for eligibility, contacting the author of the report as necessary. Reference lists of all potentially eligible articles and reviews were also searched.

A data extraction sheet was developed, and pilot tested on a random selection of included studies and refined accordingly. As many of the studies were not directly comparable (e.g. due to

different concentrations, formulations, or combinations of insecticides) a meta-analysis was not attempted. The review protocol was registered with the International Prospective Register of Systematic Reviews (CRD42016046772).

2.3 Results

Search results

The search results are illustrated in Figure 2.1. Initially 1,352 records were identified through database searches and 17 additional records were identified through other sources. After screening of title and abstracts, the remaining 108 papers were assessed and reviewed in full, after which 17 articles were excluded. The most common reasons for exclusion was that PPF only data from previous publications were included or PPF was not the main focus of the paper and no useful data were reported. A total of 91 studies were then included in the review.

Study characteristics

The included studies were published between 1989 and 2018. Six studies were written in Spanish, and the others in English. The studies came from many regions including South America (30%), North America (28%), Asia (17%), Europe (9%), Caribbean (17%), Middle east (5%), and Australia (3%). Out of all the 91 studies included, 72 (79.1%) were related to one of the following four core topics and one was related to two of the core topics:

- Efficacy of PPF granules (30 studies) [16–45];
- Auto-dissemination or horizontal transfer of PPF (19 studies) [37,46–64];
- Use of PPF ultra low volume, thermal fogging, and fumigant technologies (15 studies) [65–78];
- Assessing resistance of *Aedes* mosquitoes to PPF (10 studies) [79–88].

Other less common topics were: the use of PPF in novel products (bed nets [63,89], paints [90], release blocks [91], sugar baits [92,93], candles [94], topical treatments [95–97] ovitraps [98,99], resin sticks [100–102], and controlled release mesh [103]; the effect of PPF on the termination of the diapause process [104]; and PPF's environmental persistence and effect on non-target organisms [105]. There was also a review written in 2008 by a PPF manufacturer that focused on the different uses for PPF as a larvicide against nuisance mosquitoes and vectors of dengue and malaria [106].

Efficacy of PPF granules

PPF granules have been shown to be efficacious in a wide range of lab and field tests in countries across the world. Most studies showed Inhibition of Emergence (IE) near 100% for 90 days at higher concentrations (1-10 parts-per-million (ppm)), and a steady reduction with time post-treatment or with decreasing concentration of active ingredient (Figure 2.2). Vythilingam et al. found that adult emergence was completely inhibited for four months even with removal and addition of water [30]. However, Richie et al. found that residual PPF detected in water one week later represented just 1.2-1.4% of the total doses applied regardless of the concentration, and the authors highlighted the need to integrate the quick deterioration into any concentration planned for vector management programs [23]. Berti et al. found that increasing the number of larvae treated at 0.05 ppm did not decrease mortality of pupae or adult IE [39].

Studies also suggested that the use of PPF as an alternative to other commonly used insecticides such as temephos or in an integrated method with other means of mosquito control will increase the efficacy with subsequent reduction in the development of resistance [42,81]. Darriet et al. [40] showed the synergetic effect of the rapid killing of mosquito larvae by spinosad (an

insecticide based on compounds found in the bacterial species *Saccharopolyspora spinosa*) along with the ability of PPF to kill any pupae that emerged in their trial. Using PPF in combination with other vector control tools (Aquatain© AMF or larvicidal oil) was also suggested for emergency control programs [31]. Even using very low doses for short periods has been suggested as a strategy to reduce wild populations before the introduction of genetically modified mosquitoes [23].

Auto-dissemination or horizontal transfer of PPF

Auto-dissemination or horizontal transfer of PPF is the concept that exploits female mosquitoes to transfer lethal concentrations of an IGR to breeding or resting sites during oviposition, resulting in a reduction of mosquito population [46–49,57–64]. The possibility was first tested by forcing adult female and male mosquitoes into contact with PPF coated surfaces in the laboratory [47,58,61]. Studies showed that auto-dissemination occurred, and it successfully increased the mortality rate of larvae that were exposed (Figure 2.3).

Devine et al. [59] tested the theory in the field by distributing 1-liter plastic pots lined with damp black cloth dusted with pulverized PPF granules and achieved overall reductions in adult emergence of 42-98% thus achieving high coverage of aquatic mosquito habitats. Around the same time, Suman et al. [49], trialled the ability of mosquitoes to auto-disseminate PPF from Ultra-Low Volume (ULV) surface treatments and achieved 15.8% pupae mortality from six weeks in the first year and 1.4% pupae mortality in the second year. ULV spraying is defined as spraying of pesticides at a volume application rate of less than 5 litres/hectare to provide maximum efficacy in killing target vector mosquitoes. Both authors detected that auto-dissemination occurred, however ULV applications were determined not suitable for auto-

dissemination. Similar results were found in more recent studies using PPF sprays which found no difference in sentinel containers between intervention and control areas [54]. Better formulations and delivery methods that could load higher doses of PPF and last longer were tested [57,60] with varying degrees of efficacy.

Abad-Franch et al. used these auto-dissemination stations in a field trial and found greater than ten-fold rise and greater than a ten-fold decrease in juvenile mosquito mortality and adult mosquito emergence, respectively [46]. However, many of the field studies lacked assays sufficiently sensitive to detect the parts-per-billion concentrations of PPF, therefore limiting the direct evidence of PPF contamination [46]. However, a recent paper reporting on five different studies done in New Jersey, USA was able to detect PPF by residue analysis in field samples confirming the transfer of PPF by mosquitoes for up to 200 meters [55]. The study used auto-dissemination stations in different contexts and environments and found the stations performed effectively for 8-12 weeks and were able to contaminate 40% of sentinel containers in tire piles 50% in a junkyard. This resulted in the highest pupal mortality in peri-domestic habitats (50%), and sites contaminated with PPF 82%, although the efficacy reduced over time [55].

Snetselaar et al. [48] found 100% IE with the use of a black polyethylene device (In2Care mosquito trap) coated with PPF dust and *Beauveria bassiana*. A subsequent semi-field study using the product in Florida found the trap to be attractive to gravid mosquitoes, ability to transfer PPF to sentinel containers, reduce emergence of adult mosquitoes, and reduce survivorship of adult mosquitoes exposed to *B. bassiana* [51].

Main et al. evaluated the use of the "Auto-Dissemination Augmented by Males" (ADAM) approach which used a black plastic device to attract adult females, but also introduces directly treated males (who were exposed to PPF by an insufflator for approximately 5 sec) to transfer PPF to both larval sites and uninfected females during oviposition. Results showed 50% reduction in immature mortality rates compared with controls [62]. However, the decrease was not as large as shown by Abad-French et al., which may be due to different environments, susceptibility of the vectors to PPF, different mosquito targets, or different PPF sources and concentrations.

The benefit of auto-dissemination is the potential to effectively counter the main challenge to conventional larviciding approaches by targeting the myriad of cryptic breeding sites that these mosquitoes utilize [46,57]. However, area-wide use requires large amounts of labour when deploying and maintaining numerous stations [61]. Lastly, auto-dissemination efficacy can be affected by several factors such as insecticide resistance, coverage of treated areas, treatment methods, geographical variations and rainfall [49].

Use of PPF ultra-low volume spray, thermal fogging, and fumigant technologies

Studies investigating the use of PPF in ULV, thermal fogging (TF) and fumigant techniques found IE declining from 100%-50% for 4-6 weeks respectively in treated areas and steadily decreasing with the distance from the sprayer, the length of time from treatment, and type (cold/thermal) of fogging (Figure 2.4) [65,66,71–77]. Beyond simply having an effect on larval mortality, the sublethal dose of PPF was found to have effects on the fertility and fecundity of adult females. Therefore, even if the lethal dose is not achieved, treatment over the long-term help decrease the mosquito population through the effects on their reproductive capabilities [74].

Harburguer et al. suggested a strategy including fumigant tablets placed indoors and mixed ULV formulation (including permethrin) for outdoor application [71]. The fumigant they developed showed a high level of recovery of PPF in fumes and resulted in high levels of IE even at low concentrations, as well as an effective knockdown of adults from the permethrin. One limitation of the study data presented above was that they treated only a reduced area (200 houses in each of three different treatment areas) and there could have been infestation from adults in nearby households [71].

More recently, studies in Thailand and the USA have shown that multiple spraying machines using combinations of insecticides including PPF were unable to achieve high mortality among *Aedes* mosquitoes placed in hidden (protected) cages, and that the ULV sprays provided better emergence inhibition than the thermal foggers likely due to larger droplet size [68–70].

Assessing resistance and dose-response relationship of *Aedes* mosquitoes to PPF

Understanding resistance profiles of juvenile and adult mosquitoes is key in public health control programs. Numerous papers reviewed the susceptibility of *Aedes* to PPF and examined cross resistance among PPF and other insecticides (especially temephos). Data show IE levels of 70-100% for 250 days among higher concentrations with levels decreasing with lower concentrations and extended post-treatment time (Figure 2.5) [36,79–81,83–85,107].

Even among temephos-resistant mosquito populations IE levels show susceptibility to PPF at higher concentrations with the exception of a Florida population already resistant to two other Insect Growth Regulators (IGRs) and dichlorodiphenyltrichloroethane (DDT) [84]. However,

their data showed standard larvicides and pyrethroids used for mosquito control were still effective [84]. Indeed, this is opposite of most other studies reviewed here showing resistance of *Aedes* populations to standard larvicides and pyrethroids and susceptibility to IGRs.

Rodriguez Coto et al. [108] and Teran Zavala et al. [85] both showed that temephos-resistant strains had similar resistance ratios to reference strains and worked well even at concentrations below World Health Organization (WHO) recommended levels (Resistance Ratio is the measure of resistance in an insect population, calculated by dividing the lethal dose of a study population by the lethal dose of the susceptible population) However, three other studies [81,107,108] found that while mosquito populations were still susceptible to PPF, the lethal concentrations increased among temephos resistant mosquitoes compared to reference strains. Marcombe et al. [84] noted that as PPF has never been used in public health programs in the United States, it is possible the cross-tolerance of mosquito larvae to IGRs has arisen through the extensive use of temephos for vector control.

Safety

PPF has a very favourable mammalian toxicity profile [47]. Even treatment of drinking water at a dosage of 0.01 ppm may be used, which is 30,000 times the lethal dose for mosquitoes and six times the recommended field application rate [30,109,110]. However, as with any chemical there are still concerns regarding environmental impact of the long-term application of PPF in permanent water bodies highlighting the need for environmental studies supporting such uses [11].

2.4 Discussion

The results of this systematic review, which we believe to be the most comprehensive to date including lab and semi-field data, suggest that PPF can effectively control the emergence of adult *Aedes* mosquitoes across a wide variety of environments and in a variety of forms (e.g. granules, ULV sprays, TF, and fumigants). Utilizing a product with a favourable safety profile is especially important in settings where dose recommendations may not always be followed strictly.

Unsurprisingly, the results show the most common use of PPF in granule form results in near 100% (IE) for 90 days at higher concentrations even with removal and addition of water and regardless of the larval density [39]. Integrating PPF with other means of mosquito control (e.g. spinosad) can increase the efficacy with reduction of the risk of resistance development [111,112]. In areas where the main sources of larval biomass are identifiable and accessible, such as in rural areas with large water storage jars, controlled release PPF granules or matrixes could be quite effective.

Although PPF works well in large water containers, other cryptic or subterranean breeding sites may require significant additional work of control teams to reach. One potential solution is to utilize auto-dissemination or horizontal transfer of PPF. Evidence shows that auto-dissemination occurs, and it successfully increases the mortality rate of larvae that were exposed while reducing the number and viability of eggs from exposed females. Field trials suggest that PPF can increase juvenile mosquito mortality and reduce adult mosquito emergence, however the effect tends to reduce over time, and it is still low enough that additional tools may need to be

used in combination (e.g. granules or controlled release devices for key containers). Significant work has been published on this topic the past two or years illuminating the preferable methods of employment, and the design and spacing of devices. However, there are still no WHO prequalified auto-dissemination devices that can be purchased at large scale for control programs to use, even were they to be recommended. Future studies should look further at defining optimum design of devices and standardized approach for application of PPF dust.

In areas where *Aedes* breeding is located in large outdoor areas where key containers are not present or easy to identify, the use of PPF in ULV, TF and fumigants may be appropriate. Results show IE in treated areas near 100% and steadily decreasing with the distance from the sprayer, the length of time from treatment, and type (cold/thermal) of fogging. Sublethal doses of PPF were also found to have effects on the fertility and fecundity of adult females, suggesting positive effects may reach greater distances away from the sprayer.

Regardless of how effective different PPF products are at distributing the active ingredient, effectiveness can be reduced or lost if the mosquito develops resistance. The results suggest that even among temephos-resistant mosquito populations IE levels show susceptibility to PPF at higher concentrations, with the exception of one Florida population [84]. Many studies found that while mosquito populations were still susceptible to PPF, the lethal concentrations increased among temephos-resistant mosquitoes compared to reference strains. This is even true in areas where PPF has not been used, suggesting the possible cross-tolerance of mosquito larvae to IGRs has arisen through the extensive use of temephos. Therefore, in areas where there is already increased resistance to PPF, control programs should consider combining insecticides that work

in synergy. Regular entomological surveillance to monitor the susceptibility status of *Aedes* mosquitoes can help provide evidence and prevent development of resistance [81].

One of the limitations reported is the issue of compliance by community in areas where top-down government control programs are not distributing PPF [12]. This is due to false perceptions by the community that PPF is ineffective as it mainly acts on late instars and people may continue to observe live early instar larvae [113]. Qualitative studies are required to better understand what communication methods and materials would be most effective to increase community participation in vector control activities.

One of the limitations of this review is the intentionally broad scope and focus on the effect of PPF on *Aedes* rather than the community effectiveness of PPF products on the reduction of dengue. However, a recent review of the community effectiveness of pyriproxyfen as a dengue vector control method found "community participation and acceptance has not consistently been successful and needs to be further assessed. While all studies measured entomological endpoints, only two studies measured the reduction in human dengue cases, with inconclusive results*."* [12]

Future studies can focus on further evaluating new PPF products and new use cases for established products. It will also be important to understand the effectiveness of these products in Africa. The majority of studies represented here come from Central/South America and Asia, and none from India or Africa. However, global estimates suggest Africa's dengue burden to be equivalent to that of the Americas (16%) and together Africa and India contribute 50% of dengue cases [3]. It will be important to document the effectiveness of these products in these highly endemic areas [12].

In conclusion, the evidence for the effectiveness of PPF to increase *Aedes* larval mortality and IE is strong and consistent. However, the strength of the evidence of different product formulation and use cases varies considerably. PPF granules have highly documented and consistent results that suggest it is very effective especially when used in slightly higher doses and distributed every 30-40 weeks. The use of PPF dust for auto-dissemination and the use of PPF in ULV, TF and fumigants are encouraging although the evidence in favor of them is not as strong or consistent. Many additional novel products have been evaluated (e.g. bed nets, paints, candles, ovitraps), however evidence for these products is very weak at the moment. Future research should focus on these areas where the evidence is less strong and include additional use cases that may become developed in the future. Additional research is also needed to elucidate the biological mechanisms of cross-resistance between PPF, temephos, and other insecticides to allow control teams to make better informed decisions on which products to recommend and procure.

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Figure 2. 1: Flowchart of systematic review search process

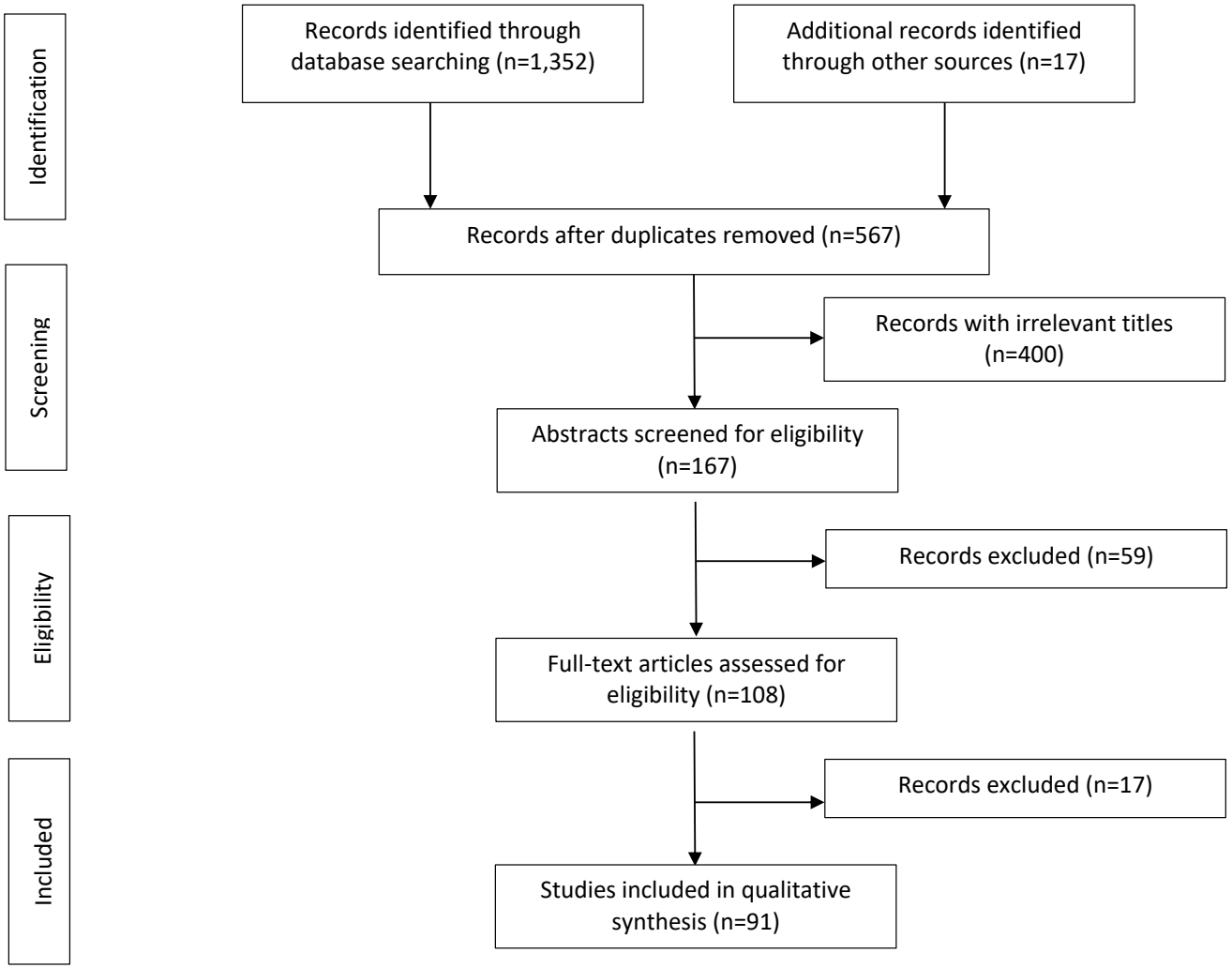

Figure 2. 2: Summary of 30 studies investigating the effect of PPF granules

First Author	Year	Country	Study Type	Product/Concentration (PPM)	Combination	% Larval Mortality	% Inhibition of Emergence (Concentration in ppm, Time Post-Treatment)	Other
Al-Azab	2013	Saudi Arabia	Lab	Sumilarv® 0.5G (NA)	Diflubenzuron	5-18%	-	
Al-Ghamdi	2008	Saudi Arabia	Lab	Sumilarv® 0.5G (0.001, 0.01)	Baycidal (IGR)	-	20% (0.001), 80% (0.01)	
Al-Solami	2014	Saudi Arabia	Lab	Sumilarv® 0.5G (0.002, 0.02)	None	10-24%	24.7(0.002) - 89.2 (0.02)	
Ali	1995	USA	Lab	TG 97% PPF (NA)	None	50% (0.00011 ppm); 90% (0.000376)	-	
Berti	2013	Venezuela	Lab	Sumilarv® 0.5G (000.2, 0.01)	None	-	77% (N/A, 8 weeks)	
Da Silva	2018	Brazil	Field	Sumilarv® 0.5G (0.01)	Grass Infusion	-	-	70 Egg Denstity Index, 120 in Control
Darriet	2006	France	Lab	TG 98.6% PPF (NA)	Spinosad	90% (PPF alone) 100% (Combination)	-	
De Resende	2006	Brazil	Lab	Sumilarv® 0.5G (0.01,0.05)	None	-	41-98% (0.01, 90 Days) 97.5-100% (0.05, 90 Days)	
Kamal	2010	Saudi Arabia	Lab	Sumilarv® 0.5G (0.02)	Diflubenzuron	14%	91.3% (0.02)	
Khan	2016	Pakistan	Lab	Sumilarv® 0.5G (0.01-0.05) & Sumilarv® 1.0G (0.01-0.05)	None	16%-78%	78% -100% (0.01-0.05)	
Lee	2001	South Korea	Field	Sumilarv 0.5G® (0.01 - 0.5)	None	-	61-96% (0.01, 70 days) 100% (0.05, 70 days)	
Loh	1989	Malaysia	Lab	TG 96.2% PPF (0.00004-0.01)	None	1.4 - 6.7%	5.7% (0.00004, 6 hours) 100% (0.01, 6 hours)	
Marina	2018	Mexico	Lab	Knack CS, 11.2% a.i. (synegenta)	None	50% (0.020 ppm)	-	0% positive ovitraps until 7 weeks in dry season (5 weeks in wet season) about 50% by week 12
			Field			-	-	
Mehmood	2015	Pakistan	Lab	Predator 0.5® (0.01)	None	-	100% (90 days) - 16% (120 Days),	
			Field			-	99-100% (45 days) - 1.1% (60 days)	
Morales	1997	Japan	Field	NA (0.1, 1, and 10)	None	-	47% (0.1), 95.2% (1), 100% (10)	
Nayar	2002	USA	Lab	Sumilarv 0.5G® (0.02, 0.05)	None	-	100%/100% (0.2/0.5, 6 Weeks)	
			Field			-	100%/100% (0.2/0.5, 6 Weeks)	
Ocampo	2014	Colombia	Field	NA - (50)	None	-	100%	
Ochipinti	2014	Venezuela	Lab	Sumilarv® 0.5G (0.01, 0.02, 0.03, 0.04, and 0.05)	None	-	78.-91.8 (N/A, 90 Days)	
Overgaard	2016	Colombia	Field	Sumilarv 0.5G® (NA)	None	-	-	Together with deltamethrin treated curtains and jar covers/lids no effect on adult index, but reduction in breteau index compared to control
Ritchie	2013	Australia	Lab	Sumilarv® 0.5G (0.1, 1, 10, and 100)	None	-	100% (100, 0-40 days), 100% (10, 0-8 days), 100% (1, 0-4 days), 45% (0.1, 2 days)	
Romeo	2009	Italy	Field	Sumilarv 0.5G® (NA)	None	-	70-100% (N/A, 5 weeks)	
Sallehudin	2004	Malaysia	Lab	Sumilarv® 0.5G (1 and 5)	None	-	100% (1, 22-28 Days) 100% (5, 36-42 Days) 90% (1, 43-49 Days) 90% (5, 64-70 Days)	
Satho	2002	Japan	Lab	TG 99% (0.0001, .0001, 0.001)	None	-	Agypti (Tanzania) - 2-3% (0.00001), 17-36% (0.0001), 58-94% (0.001) Albo (Japan1) - 11-27% (0.00001) 52- 66% (0.0001), 91-98% (0.001) Albo (Japan2) - 23-30% (0.00001) 41 - 77% (0.0001), 86-88%(0.001)	
Seccacini	2008	Argentina	Lab	97.8% PPF - 0.1% sand, 1% surfactant	None	-	100% (N/A, 45 Days) - 80% (N/A, 180 Days)	
Suarez	2011	Venezuela	Lab	Sumilarv® 0.5G (0.01, 0.05)	None	-	66-73.5% (0.01, 4 Weeks) 77-95.7, (0.05, 4 Weeks)	
Tuten	2016	Switzerland	Lab	5% I.N.D.I.A. (0.01, 0.5, 2.5, 5)	None	-	74% (0.01), 83% (0.5), 86% (2.5), 92% (5)	
Vythilingam	2005	Venezuela	Lab	Sumilarv® 0.5G (0.01, 0.02)	None	-	100% (0.1, 4 Months), 100% (0.2, 4 Months), 40% (0.2, 6 Months)	
Wang	2013	Taiwan	Lab	Sumilarv® 0.5G (NA)	None	-	100% (N/A, 14 Days)	
Webb	2012	Australia	Lab	Sumilarv® 90CS (NA)	None	-	100% (10), 20% (1)	
Xu	2010	China	Lab	Sumilarv® 0.5 (0.06, 0.12)	None	-	100%	
			Semi-field				99%	

Figure 2. 3: Summary of 19 studies investigating the effect of auto dissemination or horizontal transfer of PPF

Reference	Year	Country	Type of Study	Product/concentration (g/m2)	# of Devices	% Larval Mortality (Time)	% Inhibition of Emergence	Other
Abad-Franch	2015	Brazil	Field	Sumilarv® 0.5G (5)	100	90%	-	
Buckner	2017	USA	Semi-Field	In2Care	5	-	Intervention: 80% (agypti) 90% (albo) ; Control: 20%-30%	
Caputo	2012	Italy	Lab	Sumilarv® 0.5G (0.5)	10	20%	-	
		Italy	Lab	Sumilarv® 0.5G (5)	10	50-71%	-	
Chandel	2016	USA	Semi-Field/open	TG PPF (60% a.i.)	4	15% (mean - 8 weeks in 2013)/ 30% (mean 12 weeks 2014)	-	
			Semi-Field/cryptic	TG PPF (60% a.i.)	8	10% (mean - 8 weeks in 2013)/ 10% (mean 12 weeks 2014)	-	
Chism	2003	USA	Lab	TG PPF (0.3)	-	10-30%	-	
		USA	Field	TG PPF (0.4)	-	50-90%	-	
Devine	2009	Peru	Field	Sumilarv® 0.5G (5)	30	50% - 80% (Two Sites)	-	
Gaugler	2012	USA	Lab	TG PPF/NyGuard® (NA)	-	-	Cages-100%; Small Room-80%	
Itoh	1994	Thailand	Lab	95.2% TG PPF (1.0)	-	-	23%-95%; Control 3%	
Kartzinel	2016	USA	Lab	Esteem® (NA)	2	45% inervention - 1% control	-	
			Field	Esteem® (NA)	20	Site 1 (1%), Site 2 (4-30%, Site 3 (0-12%), Site 4 (0-10%)	-	
Llyod	2017	USA	Field	Nyguard® - 10% PPF	-	-	-	Overall, there were no differences in pupal mortality between the control and autodissemination vases
Mains	2015	USA	Field	Esteem® 35 WP IGR/DayGlo® (NA)	-	70% (Female Experiment) 95% (Male Experiment)	-	
Ohba	2013	Japan	Semi-Field	TG Sumlarv® 1.0% (w/v) (0.35)	-	Intervention: 50% (20 Days), Control 20% (20 Days)	-	
		Japan	Semi-Field	TG Sumlarv® 0.1% (w/v) (0.035)	-	50% (6 Days), Control 0% (6 Days)	-	
Ponlawat	2013	Thailand	Semi-Field	Sumilarv® 0.5G (0.05)	-	-	25% treated, 10% control	
			Field		4	-	-	The post-treatment rate ratio (0.4) for treatment area indicated the pyriproxyfen-treated device significantly reduced adult counts during the study period.
Sihuincha	2005	Peru	Field	Sumilarv® 0.5G (NA)	-	75%	-	
		Peru	Lab	Sumilarv® 0.5 G - 50, 67, and 83 ppb (direct application)	-	100% (5 Months)	-	
Suman	2014	USA	Field	NyGuard® (NA")	-		-	The sentinel containers for autodissemination showed 15.8% pupal mortality (week 1–6) in the first year, and 1.4% pupal mortality in the second year. No significant difference was detected among the distances and direction for pupal mortality.
Suman	2018	USA	Field (Essex) -2012	TG PPF (60% a.i.) - MGK®	6	-	-	Pupae Mortality of 15-20% over 12 weeks compared to 3% in Control
			Field (Hudson) - 2014		24	-	-	Pupae Mortality of 13.9-20.3% over 8 weeks compared to 1% in Control
			Field (Hudson) - 2012		1,2,4	-	-	Pupae Mortality of 10-25% over 12 weeks compared to 5% in Control
			Field (Middlesex) - 2013		1	-	-	Pupae Mortality of 50.4% over 8 weeks compared to 5% in Control
			Field (Mercer) -2012		1	-	-	Pupae Mortality of 5-10% over 12 weeks compared to 2% in Control
Snetselaar	2014	Netherlands	Lab	NA	4	-	95%; Control 2%	
Tuten	2016	Switzerland	Semi-Field	5% PPF powder - I.N.D.I.A.	5	-	-	3 of 4 trials had staticailly significant difference in pupae between intervention/control
Unlu	2017	USA	Field	20% PPF oil & 60% powder	81	-	-	Pupal Mortality 12.4% control 0.58% after 50 days, no difference in adults

Figure 2. 4: Summary of 15 studies investigating the effect of PPF ULV spray, thermal fogging, and fumigant technologies

Reference	Year	Country	Type of Study	Product/Concentration	Combination	% Larval Mortality	% Inhibition of Emergence (Time-Post Treatment)	comment
Dantur Juri	2013	Argentina	Field	ULV treatment - 3% PPF, Fumigant - 0.2% PPF	Permethrin	100%	-	
Doud	2014	USA	Field	Nyguard®- 10% PPF	None	81.6%-87.4%	93.13-97.97%	
Harburguer	2011	Argentina	Field	ULV treatment - 2% PPF, Fumigant - 2% PPF	Permethrin, Methyl 3	95.5%	92.60%	
Harburguer	2011	Argentina	Field	Fumigant - 2% PPF	Permethrin	100%	89.50%	
Harburguer	2012	Argentina	Field	ULV treatment - 2% PPF, Fumigant - 2% PPF	Permethrin	-	47-52% Inside, 59.2-71.0% Outside	
Harburger	2014	Argentina	Lab	0.2 g/kg PPF	None	-	20%	
		Argentina	Lab	2 g/kg PPF	None	-	40%	
Harburger	2009	Argentina	Confirmatory	2 g/kg PPF	Permethrin	-	95-97% (30 min)	
Harwood	2016	USA	Field	Nyguard®- 10% PPF	ULB BP-300	-	-	Sprayers producing larger droplets (misters and cold foggers) were more effective in controlling immature mosquitoes indoors and outdoors. Thermal fogging was more effective in controlling adults indoors, whereas cold fogs and misters were more effective for outdoor control
Harwood	2014	USA	Semi-Field	Nyguard®- 10% PPF	ULB BP-300	-	ULV - 50-80% (0-4 Weeks), TF 25-50% (0-4 Weeks)	
Lloyd	2017	USA	Field	Nyguard®- 10% PPF	None	-	-	The tire pile samples had significantly more mortality (P , 0.0001) out to 4 wk when compared to autodissemination and control vases.
Lucia	2009	Argentina	Field	3% PPF	Permethrin	-	Days)	
Ponlawat	2017	Thailand	Field 1 (Patriot)	Nyguard®- 10% PPF	ULB BP-300	-	3.94-21.33 (1 day); -3.35-12.10 (7 days), 1.55-19.78 (14 days)	
			Field 1 (Twister)			-	-4.72-100 (1 day); 1.2-99.29 (7 days), -4.83-97.27 (14 days)	
			Field 1 (Patriot)			-	-9.8-99.57 (1 day); -10.25-68.08 (7 days), -2.99-67.47 (14 days)	
Fiorenzano	2013	USA	Semi-Field	Nyguard®- 10% PPF	None	-	Direct Treatment -50-100%; Indirect -70%-100%	
Unlu	2018	USA	Field	Archer IGR - 1.3% PPF	Al lambda-cyhalothrin	-	-	Applications resulted in significant decreases in adult mosquito abundance post- treatment of 74% compared with the untreated control. Both insecticides exceeded the 70% reduction threshold considered as effective for Ae. albopictus control for 2 to 4 wk. However, applications of Archer IGR alone did not reduce adult mosquito abundance.
Unlu	2018	USA	Field	Nyguard®- 10% PPF	Sumithrin, prallethrin, and *Bti*	-	-	The adult emergence inhibition was significantly higher in the treatment bioassay cups t (z=4.65, P<0.0001) and field control bioassay cups (z=8.93, P<0.0001) than controls. They observed a lower trend in adult numbers following the seasonal long combined application of pyriproxyfen and adulticide, with numbers of adult Ae. albopictus in the treatment site up to five times lower than in the control site.

Figure 2. 5: Summary of 10 studies investigating resistance of *Aedes* mosquitoes to PPF

Reference	Year	Country	Type of Study	Product/Concentration (ppm)	Additional Prodcuts Used for Comparison with PPF	Resistance Ratios (Lethal Dose Suceptable / Resistant Strains)	% Inhibition of Emergence (Concentration in ppm, Time Post-Treatment)
Adrighetti	2008	Brazil	Lab/Semi-Field	TG 98.5%/Sumilarv® 0.5G (0.05)	Temefós Fersol 1G	1.4-6.5	100% (N/A,44 Days)
Darriet	2010	Martinique	Semi-Field	Sumilarv® 0.5G (0.02)	Spinosad	-	80% (N/A, 150 days), Combination - 80% (N/A, 250 days)
		Martinique	Field	Sumilarv® 0.5G (0.02)	Spinosad	-	80% (N/A, 21 days), Combination - 80% (N/A, 126 Days)
Gomez	2011	Argentina	Lab	TG 97.8% (NA)	Temephos, *Bti* , Permethrin	-	50% (0.01642-0.00774)
Lau	2015	Malaysia	Lab	Sumilarv® 0.5G (NA)	None	1.4	-
Lau	2018	Malaysia	Lab		Methoprene, Difubenzuron, Novaluron, Cyromazine	0.09	-
Leyva	2010	Cuba	Field	97% PPF (NA)	None	0.5-3.4	-
Marcombe	2011	Martinique	Lab	TG 98.7% (NA)	*Bti* , Temephos, Spinosad, and Diflubenzuron	2.2	-
		Martinique	Semi-Field/Field	Sumliarv® 0.5G (0.2, 0.5)	*Bti* , Temephos, Spinosad, and Diflubenzuron	-	Semi-field - 80% (0.05, 250 days), 80% (0.02, 160 days), Field - 80%
Marcombe	2014	USA	Lab	TG 99.1% (NA)	*Bti* , Temephos, Propoxur, Spinosad, Methoprene	0.38-2.36	-
Leyva	2013	Cuba	Lab	TG 97% (NA)	None	-	30-40% (1) to 100% (10)
Teran Zavala	2014	Ecuador	Lab	TG 97% (NA)	Temephos	4.2-9.2	Temephos Resistant - 40% (1), 100% (10); Susceptible - 100% (0.1-50)

*Additional information on comparisons and resistance ratios can be found on page 50

Chapter 3: Determining the efficacy of guppies, pyriproxyfen (Sumilarv® 2MR), and community engagement on dengue vectors in Cambodia: a study protocol for a cluster randomized trial

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

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SECTION A – Student Details

Student ID Number	Lsh1406618	**Title**	Mr
First Name(s)	John Christian		
Surname/Family Name	Hustedt		
Thesis Title	Determining Effectiveness of New Approaches to Dengue Vector Control in Cambodia		
Primary Supervisor	john.hustedt@lshtm.ac.uk		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Trials		
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	This PhD is centred on a cluster-randomized trial of which I led the conceptualization, design, and protocol-writing with Jeffrey Hii. I was primarily responsible for writing the SOPs, training materials, and field manuals. I was also responsible for all activities of the trial in Cambodia during its execution including supervising all data collection, entry, and management. I also completed all data analysis and wrote reports to the Technical Steering Committee, ethics committees, and donors. I was a Co-Investigator, and Senior Technical Officer with the Malaria Consortium, for the trial. Members of the PhD Advisory Committee, including my two LSHTM supervisors and Jeffrey Hii — the Principle Investigator of the trial, based in Thailand — provided substantial guidance, including suggested edits to the protocol and other documents.

SECTION E

Student Signature	John Hustedt
Date	July 21, 2019

Supervisor Signature	[redacted]
Date	25 July 2019

Determining the efficacy of guppies and pyriproxyfen (Sumilarv® 2MR) combined with community engagement on dengue vectors in Cambodia: study protocol for a randomized controlled trial

John Hustedt[1,2*], Dyna Doum[1], Vanney Keo[1], Ly Sokha[3,] BunLeng Sam[3], Chan Vibol[4] , Neal Alexander[2], John Bradley[2], Marco Liverani[2], Didot Budi Prasetyo[5,] Agus Rachmat[5], Muhammad Shafique[1], Sergio Lopes[1], Leang Rithea[3], Jeffrey Hii[1]

*corresponding author

Author's information

[1] Malaria Consortium (House # 91, St. 95,Boeung Trabek, Chamkar Morn; PO Box 2116, Phnom Penh 12305, Cambodia)

[2] London School of Hygiene and Tropical Medicine (Keppel Street, London, WC1E 7HT, United Kingdom)

[3] Cambodian National Dengue Control Program (372 Monivong Boulevard, Phnom Penh, 12302)

[4] World Health Organization (No. 177-179 corner Streets Pasteur (51) and 254; Sankat Chak Tomouk Khan Daun Penh, Phnom Penh, Cambodia)

[5] US Naval Medical Research Unit-2 (#2, St. 289, Boeung Kok 2 commnue, Toul Kork district,, 289 Samdach Penn Nouth, Phnom Penh 1225

Abstract

Background: Evidence on the effectiveness of low-cost, sustainable biological vector control tools for *Aedes* mosquitoes is limited. Therefore, the purpose of this trial is to estimate the impact of guppy fish, in combination with the use of the larvicide Pyriproxyfen (Sumilarv® 2MR), and Communication for Behavioural Impact (COMBI) activities to reduce entomological indices in Cambodia.

Methods/Design: In this cluster randomized, controlled superiority trial, 30 clusters comprising of one or more villages each (with approximately 170 households) will be allocated, in a 1:1:1 ratio, to receive either a) three interventions (guppies, Sumilarv® 2MR, and COMBI activities), b) two interventions (guppies and COMBI activities), or c) control (standard vector control). Households will be invited to participate, and entomology surveys among 40 randomly selected households per cluster will be carried out quarterly. The primary outcome will be the population density of adult female *Aedes* mosquitoes (i.e. number per house) trapped using adult resting collections. Secondary outcome measures will include the House index, Container index, Breteau index, Pupae Per House, Pupae Per Person, mosquito infection rate, guppy fish coverage, Sumilarv® 2MR coverage, and percentage of respondents with knowledge about *Aedes* mosquitoes causing dengue. In the primary analysis, adult female *Aedes* density and mosquito infection rates will be aggregated over follow-up time points to give a single rate per cluster. This will be analysed by negative binomial regression, yielding density ratios.

Discussion: This trial is expected to provide robust estimates of the intervention effect. A rigorous evaluation of these vector control interventions is vital to developing an evidence-based dengue control strategy and to help direct government resources.

Trial Registration: Current Controlled Trials ISRCTN85307778; October 25, 2015

Key Words: Dengue, Guppy, Pyriproxyfen, Community Engagement, Vector Control, Cambodia

3.1 Introduction

Dengue is one of the most rapidly spreading mosquito-borne viral disease in the world, and is caused by bites of infected *Aedes* mosquitoes, principally *Aedes aegypti* [1]. Dengue infection is a systemic and dynamic disease with a wide clinical spectrum that includes both severe and non-severe manifestations and, in some cases, can lead to death [1]. With an estimated 3.6 billion people in 124 countries at risk of contracting the disease [2] and 390 million dengue infections occurring each year (of which 96 million are clinically apparent) [3] the dengue virus has become a leading cause of illness in the tropics and subtropics [4]. Asia records 70% of the global disease burden due to dengue [3], and Cambodia has one of the highest per-capita incidence rates in the region [5].

Identified in Cambodia in 1963 [6], a total of 194,726 dengue cases were reported to the National Dengue Control Program (NDCP) between 1980 and 2008 [7]. Between 2003 and 2008, annual dengue incidence ranged between 0.7 and 3.0 per 1,000 persons, the cost to society estimated at between $3,327,284 and $14,429,513 [8]. Since most of this cost falls onto the family, it is estimated that 67% of affected households fall into debt to pay for medical bills [9]. However, it is likely that the real number of cases and cost to society is much greater, with some studies suggesting the real case numbers are between 3.9 and 29.0 times higher than those of the National Dengue Surveillance System [10, 11].

Although a number of promising vaccine candidates are in preclinical and clinical development [12], and methods of genetic control of mosquitoes are being developed [13-15], they are years from operational roll-out in Cambodia and are unlikely to provide universal

protection. Without a cure or vaccine, the most appropriate dengue control measures are vector control and the avoidance of mosquito bites. Several vector control methods have been studied in Cambodia including chemical and biological substances (temephos, pyriproxyfen, and *Bacillus thuringiensis israelensis)* [16-19], jar covers [20], distribution of larvivorous copepods and fish [21-23].

Past research

Ae. aegypti is highly anthropophilic (preference for human beings), endophilic (resting indoors), endophagic (preference for feeding indoors) [19]. This partially explains why previous studies showed that household water storage jars contained over 80% of *Ae. aegypti* larvae in Cambodia, and why these jars became the main target for dengue vector control activities [20]. Since the early 1990s, NDCP has used the larvicide temephos (distributed with brand name Abate®) to target large (200-400L) household water containers as the primary means of vector control [19]. This has continued despite susceptibility tests in 2001 showing resistance of *Ae.* a*egypti* in urban Phnom Penh and incipient resistance in a rural province in Cambodia [24]. An evaluation of the effectiveness of temephos control programs to control larvae in 2007 concluded that control strategies emphasizing the use of temephos should be reconsidered [19]. Although temephos was only distributed during the rainy season, there were still containers found positive for immature *Aedes* during the dry season; and the program ignored discarded containers - which had twice the number of larvae as water storage containers. Khun and Manderson (2007) concluded that "continued reliance on temephos creates financial and technical problems, while its inappropriate distribution raises the possibility of larvicide resistance." [19]

Following the success of *Mesocyclops* (a genus of copepod crustaceans) programs in locally eliminating *Aedes* mosquitoes in Vietnam [25-27], the Cambodian NDCP implemented a two-year *Mesocyclops* project in Kratie province from 2002-2004, searching for an alternative vector control option [23]. Initial results showed a reduction in the *Aedes* population in the intervention area, but by the end of the project larval densities in the intervention area had increased by 62% from baseline. This apparently lower effectiveness in Cambodia may be because *Mesocyclops* from the local water sources had various parasites and colonizing them parasite-free requires special training beyond what is possible in most rural Cambodian villages. The environment could have played a role as Northern Vietnam (where programs were most successful) has four distinct seasons and has different flora and fauna. Additionally, people did not accept *Mesocyclops* to the same extent as other interventions that were provided by the NDCP such as temephos (To Setha, personal communication, 2015).

The search for other vector control options continued with an evaluation of *Bacillus thuringiensis israelensis* (*Bti),* a Gram positive, soil dwelling bacterium used a biological control agent [18]. The evaluation of *Bti* in Phnom Penh showed positive results with significant reductions in the number of pupae for at least 2 and 2.5 months in containers with river and well water, respectively [18]. More extensive usage and evaluation of *Bti* by the Cambodian government are currently occurring in Kandal and Kampong Thom Provinces (Personal communication, Bunleng Sam, 2015).

Jar covers with long-lasting insecticidal netting (LN) treated with deltamethrin were found to have significantly fewer pupae per house, a threefold decline in *Ae. aegypti* adult females per

house and adult mosquito survival [20]. However, the magnitude of the reduction diminished over time, due to a gradual reduction of insecticidal effect of the jar covers and a residual deltamethrin life of 22 weeks [20]. Interestingly, this is less than the average residual life of deltamethrin in treated bed nets [21]. Another cause may have been children not always keeping the jar covering on after extracting water and using them as toys around the house (Personal communication with To Setha, 2015) as Khun et al. noted in Cambodia before [28]. Improvements in engineering and design to prevent entry and egress of mosquitoes, especially when the container is used, and an increase the insecticidal effectiveness may be needed for jar covers to be cost-effective public health interventions [20].

The use of a larvivorous guppy fish (*Poecilia reticulata*) was evaluated in 14 Cambodian villages [21], and subsequently in a larger study of 28 Cambodian villages [22]. Results from the initial study done from 2006-2007 were extremely encouraging with guppies in 56% of eligible containers, and a 79% reduction in *Aedes* infestation compared to the control. Guppy fish are not able to colonize all potential *Aedes* breeding sites, especially those which are polluted or with volume of less than 50L (To Setha, personal communication, 2015). However, despite not having guppies, the smaller or discarded containers in the intervention villages had 51% less infestation than those in the control, suggesting a community-wide protective effect [21]. This could partly be due to spill over effect from treatment villages as no results of guppy coverage were reported in the paper. These results led the WHO and the Asian Development Bank (ADB) to fund a larger scale-up in 2010-2011 which included Communication for Behavioural Impact (COMBI) activities. Results showed 88% guppy fish coverage in eligible water containers and a Container Index or proportion of surveyed containers containing *Ae.* aegypti larvae/pupae and

indoor resting adult females of near zero (while control had a CI of 30) at the end of the project [22]. Similarly encouraging results were found in Laos as a part of the same project. However, there were additional miscellaneous breeding sites including containers too small for guppy survival. Additional tools beyond larvivorous fish are required to target these varied, hard-to-reach and cryptic breeding containers or sites.

One such alternative that has been evaluated in Cambodia is pyriproxyfen (PPF) [16, 17]. PPF is a juvenile hormone analogue that interferes with the metamorphosis of juvenile *Aedes* mosquitoes, preventing their development into adults [29]. The results of the first study in 2003 were so promising — at higher doses, inhibition of adult emergence (IE) greater than 87% for six months — that a larger second study was designed [16]. This showed that a novel 5% controlled release formation led to IE above 90% for 20 weeks, and above 80% for 34 weeks [17]. A slow-release PPF matrix release formulation (Sumilarv® 2MR) has since been developed and is suitable for containers uninhabitable by guppy fish. The added benefit of this product is that it only requires one distribution every six months (the entirety of the rainy season) and cuts down on operational costs as compared to temephos or *Bti* which have residual efficacy of 2-3 months [18, 30].

The effective implementation of Integrated Vector Management requires mobilization and coordination of the resources needed to achieve and sustain behaviour changes among populations at risk of dengue [31]. The COMBI strategy provides a social mobilization and communication approach that connects knowledge and behaviour, addresses the cost and value of engaging in healthy behaviours, recognizes the gradual stages of behaviour change, and

creates a supportive environment for behaviour change [32]. The challenge for vector control is how community participation can be integrated into vector breeding source reduction efforts [22]. Community Health Workers (CHWs) are a vital part of successful COMBI communication and social mobilization activities. A recent review of 22 studies found that educational messages embedded in a community-based vector control approach were effective at reducing *Ae. aegypti* measured through entomological indices [33]. A separate systematic review found that community-based control strategies in addition or together with biological and chemical vector control tools reduced classical *Aedes* larval indices in five of six field trials [34]. Two cluster randomized trials published after the reviews showed that a community empowerment strategy embedded in a routine dengue vector control program drastically reduced entomological indices [35, 36]. These past studies show the importance of having a strong community-based COMBI strategy, and the important contribution it can add when integrated into the vector management strategy.

Need for a trial

Although there is evidence suggesting the use of guppy fish can be beneficial in dengue vector control, recent reviews show there has never been a cluster randomized trial to evaluate their effectiveness to reduce mosquito indices [37]. Although some studies have evaluated the use of community-based communication programmes for dengue control, a recent review found that none had assessed their costs [34]. This trial has the potential to inform the strategic application of community-based distribution of Pyriproxyfen and larvivorous fish in an outbreak, during inter-epidemic periods or for broad scale application. This trial will also be the first to our knowledge to evaluate the widescale use of the new Sumilarv® 2MR product in the field

(personal communication, John Lucas, 2015). Although guppies, Pyriproxyfen (PPF), and COMBI activities have all been evaluated, some of these evaluations were methodologically flawed. Furthermore, they have never been tested in combination. Therefore, our study is intended to fill the knowledge gaps identified above.

Hypothesis

This trial aims to demonstrate community effectiveness of guppies, PPF, and COMBI activities. The main hypotheses are:

1. Use of guppies, Sumilarv® 2MR and COMBI activities will reduce numbers of *Aedes* mosquitoes, and their infection rates, more than guppies and COMBI alone, or usual ministry of health activities (including larval control and information and education material dissemination during outbreaks) as assessed through entomology surveys;
2. COMBI activities will improve the community's knowledge, attitudes, and behaviour around water use and vector borne disease prevention (such as burning or burying discarded containers, cleaning the environment around the house, and sleeping under a bed net) as assessed through baseline/endline surveys and Focus Group Discussions (FGDs);
3. Guppies and pyriproxyfen will be acceptable among the target villages as assessed by an endline survey and FGDs.

The study is designed as a cluster randomized, controlled superiority trial with three parallel arms.

3.2 Methods

This protocol is reported following the criteria of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) [38]. For the completed SPIRIT checklist see Appendix 3.1.

Study setting

The study has 30 clusters in two operational districts (ODs) (one OD includes the jurisdiction of 10 health centres (HC) or roughly 100,000-200,000 individuals) within Kampong Cham province. Each cluster has on average 200 households or 1,000 individuals. The rainy season runs from April to November, and the peak dengue season is from May-July. Kampong Cham was selected as it has one of the highest dengue incidence rates of 1.6 cases per 1000 people in Cambodia and the environmental characteristics are similar to most dengue-endemic areas of Cambodia (Personal communication, Hai Ra, 2016). The clusters (containing one or more villages) were selected based on availability of entomological surveillance data from previous surveys. To avoid spill over effects, clusters had to be at least 200 meters from the nearest household outside the cluster since *Ae. aegypti* in this region have an average flight range of 50-100m [39] (Figure 3.1).

Eligibility criteria

Every house within the cluster boundaries will be invited to participate in the trial.

Interventions

Selected villages will be randomized into one of three study arms (See Table 3.1). Study arm one receives all three interventions, while study arm two receives only guppies and COMBI

activities, and arm three will receive only the standard vector control activities from the Ministry of Health. The total trial period for the interventions will be 11 months (See Figure 3.2 and Figure 3.3).

Table 3. 1: Interventions randomized to each study arm

Intervention	Arm 1	Arm 2	Arm 3
Guppy Fish in key containers (>50L)	X	X	
COMBI activities	X	X	
Direct PPF application (Sumilarv® 2MR) in smaller containers (10-50 L)	X		

Study arm one was chosen to evaluate the effectiveness of all three interventions in combination. Application of any insecticide can be expensive when taking into account procurement and operational costs. Arm two was selected to evaluate the effectiveness of less expensive interventions (guppies and COMBI), although all strategies are expected to be less expensive than current strategies. As COMBI related activities have been shown to have a significant impact on coverage of interventions in Cambodia and elsewhere [21, 22, 34] a guppy only arm was not included. Therefore, the trial will not give separate estimates of the effects of guppies and COMBI. Larvicide only arms were not included because larvivorous fish are more sustainable and cost effective than larviciding [16, 17, 21, 22, 40, 41], and if larviciding was found to be equally effective, guppies would be recommended in terms of cost and acceptability.

Guppies

In rural Cambodia, more than 80% of the *Aedes* mosquito breeding is detected in key containers such as large water jars, cement tanks and other large containers used for the storage and collection of water for human and animal consumption and washing [20]. To target these containers, two guppy fish (*Poecilia reticulata*) will be placed into each water container greater than 50L in intervention villages (Arm 1 and 2). This is based on larval consumption of guppies determined by Seng et al. [21] and past experiences using guppies in vector control in Cambodia [22]. The guppy fish from the colony established by NDCP will be distributed after the baseline activities through a local community network managed by provincial government authorities. Guppy banks will be set up at the corresponding health centres and consist of twenty 500L jars. Guppy banks will be colonized and can provide fish at any time to CHWs in implementation villages. One CHW will be assigned to monitor 30 Households (HHs) each month. They will each have two 500L jars which they can colonize with guppies to provide for their assigned households. When CHWs need more guppies, they can return to the guppy bank for them. Each month CHWs will conduct visual checks and ensure all their assigned households have guppies in all large containers and replace them if necessary. Adult guppies are on average 1.5–3.5 cm long (males) or 3–6 cm long (females) [42].

Pyriproxyfen matrix release (Sumilarv® 2MR)

Each device or disc is designed to provide coverage for 40 L of water (Figure 3.4). It is also possible to cut discs into smaller sizes for smaller size containers (Table 3.2).

Table 3. 2: Dosage application rate of Sumilarv® 2MR discs

Container capacity, L	No. of 2MR discs	Target ppb
10	1/5	27
20	1/2	27
30	2/3	27
40	1	27
50	1	27

PPF devices will be distributed after the baseline survey in the same manner as described above and replaced after 6 months. Additional devices will be left at the health centre for CHWs to distribute during their monthly monitoring visit if some have been lost or need to be replaced.

Although there have not been any studies evaluating the safety of PPF in humans, toxicity to fish is induced at 450 ppb, which is approx. 45 times greater than the target ppb (10) of Sumilarv® 2MR [43]. The LD50 in rats is 5,000,000 ppb, or 500,000 times the target concentration [44]. These data suggest a very favourable mammalian toxicity profile, and extremely low risk for humans using this product.

Communication for behavioural impact activities

An initial rapid assessment consisting of FGDs and In-Depth Interviews (IDIs) regarding knowledge, attitudes, and behaviours of community members was completed. The results were used in a message and material development workshop held with key community and district stakeholders. During this meeting the community helped develop behaviour change

communication materials and come up with key messages. The results were used to understand the common social gathering locations for health education sessions, culturally appropriate channels of communication, and to create communication materials: flip charts to guide CHW education sessions, posters and banners for display in the villages, songs, and CHW materials such as hats, t-shirts, bags, and rain coats.

A two-day training will be given to CHWs on communication and facilitation skills, following which they will take the lead role in conducting health education sessions in their community. A monthly meeting will also be conducted with CHWs to assess progress, address issues and challenges, and provide them continuous training to develop their confidence and skills. The health education sessions will occur twice per month and will be participatory, as Khun and Manderson [28] found that health educations sessions where participants actively identify breeding sites and practice positive behaviours can be more effective and less costly than the didactic classroom-based sessions. In addition to health education sessions we will use locally available media such as loud speakers fixed to local transport to play songs, street theatre performances, and role playing to reinforce the messages.

Adherence

In order to improve adherence to the intervention protocols, CHWs will perform monthly monitoring checks on each household within the intervention arms. The presence or absence of guppy fish and PPF Sumilarv® 2MR in each container within the household will be recorded along with any replacements the CHWs provide. The entomology surveys will also record the presence or absence of each intervention in containers (including those used for domestic and

non-domestic use) within the surveyed households. Project staff will also randomly visit CHWs and intervention households to confirm the reliability of data provided.

Primary outcome measures

The primary outcome measure is the population density (i.e. number of mosquitoes per unit of time spent aspirating) of adult female *Aedes* trapped using adult resting collections.

Secondary outcome measures

The secondary outcomes for the trial include:

- Dengue virus infection rate in adult female *Aedes* mosquitoes
- House index (HI): Proportion of houses surveyed positive for *Aedes* larvae and/or pupae in any water container
- Container index (CI): Proportion of surveyed containers containing *Aedes* larvae and/or pupae
- Breteau index (BI): Number of containers positive for *Aedes* larvae and/or pupae per 100 houses surveyed
- Pupae Per House (PPH): Number of *Aedes* pupae per household
- Pupae Per Person (PPP): Number of *Aedes* pupae per person
- Guppy fish coverage: proportion of eligible water containers with ≥1 guppy fish
- Sumilarv® 2MR coverage: proportion of eligible water containers with ≥1 MR
- Percentage of respondents with knowledge about *Aedes* mosquitoes causing dengue

Sample size

The guppy fish and pyriproxyfen interventions will be assessed by an entomology survey. A sample size of 10 clusters per arm and 40 HHs per cluster for the survey was devised using the Hemming and Marsh method [45]. The distribution in each cluster is assumed to be Poisson, and the between-cluster variation is assumed to be Gaussian (normal). The calculation assumed a mean of 0.1 adult female resting *Aedes* per household in the intervention arms compared to 0.25 in the control arm for each collection. This assumption was based on the results from the earlier World Health Organization/Asian Development Bank guppy fish project in the same province [22], and to be conservative assumed no impact from the PPF in arm 1. The households will be randomly selected each collection. The intra cluster correlation (ICC) was assumed to be 0.01 based on previous studies [46-48]. Additionally, a sensitivity analysis was conducted up to the median value of ICCs for outcome variables (0.03) as found by an analysis conducted by Campbell et al [49]. Our analysis determined that we would have between 91 to 75 percent power at ICC values between 0.01 to 0.03. Under these assumptions the study will have 91-75% power to detect a difference at the 5% significance level.

COMBI activities will be evaluated through Knowledge, Attitudes, and Practice (KAP) surveys. A sample size of 10 clusters per arm and 20 HHs per cluster was devised, again using the Hemming and Marsh method [45]. The calculation assumed a 22.5% change in primary outcome indicators from 40% to 62.5% in intervention villages and no change in the control villages over the course of one year. Outcome indicators include:

- Percentage of respondents with knowledge about mosquitos causing dengue.

This calculation was based on the results from the earlier projects done by Malaria Consortium (MC) in the region, and a recent unpublished MC KAP survey completed in 6 provinces and 30 villages in 2014. In terms of variation in cluster size (as opposed to between-cluster variation in the outcome), the coefficient of variation was assumed to be 0.1 and is expected to be small as we plan to sample the same number of houses from each cluster. Although the total number of houses varies between cluster, as shown in the flowchart (Figure 3.2), the sample size is based on the number of houses sampled for *Aedes*, which is under the control of the investigators. Under these assumptions the study will have 90% power to detect a difference at the 5% significance level.

Allocation

Clusters will be randomly assigned with a 1:1:1 allocation through a public randomization process. Village chiefs from all clusters and HC chiefs from all HCs will be invited to a central point along with local and national authorities. Locally the concept of "lucky draw" is very popular, so this method will be used to randomize clusters. Each cluster representative will choose one paper labelled arm one, two, or three from a bowl. The numbers on the papers will be printed and concealed by folding the paper in half four times. Three large labelled sheets of paper were put on the wall. As each representative selected their study arm, MC staff will write the cluster names on the corresponding sheet.

Data collection methods

Data will be collected at 0, 4, 8, 12 months post-intervention, unless otherwise mentioned. The project will employ the following methods:

Entomology

A baseline survey was conducted prior to start of interventions. An endline survey will be conducted one year after the baseline. Two additional surveys during the dry season (4 months post intervention) and light rain (8 months post intervention - peak dengue season) will also be conducted. The schedule of the surveys took into account data from the previous guppy fish project [22]. The survey methodology was developed following the WHO guidelines for entomological collections [1]. Surveys will include indoor adult resting catches and larvae/pupae collection from water containers. The survey team consisted of experienced government staff who received three days training before beginning. All tools and materials were pre-tested during training. The same team will be used for each entomology survey. Houses within each cluster were selected using a random number generator [50] applied to the village list managed by the village head.

The adult resting catch will be completed using a battery-powered, portable aspirator (Camtech, Phnom Penh, Cambodia) for 10 minutes per house in the bedrooms and living spaces, starting in the bedroom and aspirating up and down the wall (from floor to 1.5 m) around the home in a clockwise manner. The mosquitos will be kept in a screw top container inside a cold box and transported to the provincial laboratory for identification to the species level for *Aedes*, otherwise to genus. All *Aedes* mosquitoes will be sexed. After identification they will be stored in a -20 degree Celsius freezer and taken to the United States Naval Medical Research Unit 2 (NAMRU-2) in Phnom Penh for confirmation. All *Aedes* females will be pooled and subjected to flavivirus

rRT-PCR screening [51]. Flavivirus positive pools will be further tested for dengue typing by semi-nested RT-PCR assay targeting the C and pre-M regions within the viral genome [52]. Larvae and pupae collection will be completed using the five-sweep net method [53] for containers larger than 50 litres. The size of the net is 20 cm by 33 cm. Surveyors will turn the net in an anti-clockwise manner 5 times, then wait 1 minute and perform one sweep from the bottom. This method can sample around 35 percent of larvae and 31 percent of pupae, and the total number estimated by an adjustment factor [53]. For containers less than 50 litres, all the water will be poured through the sweep net. All containers within selected households will be inspected. All pupae and ten larvae per container will be put in a plastic bag, labelled (with date and code), and taken back to the laboratory for identification to the species level for *Aedes*, otherwise to genus. After identification they will be taken to NAMRU-2 in Phnom Penh where entomologists will confirm identification of a random sample of 50 percent of immature mosquitoes.

Survey teams will also record the number, size, and type of all water containers in the household. The team will complete a rapid assessment tool (Premise Condition Index) (PCI) [54] to identify whether the scores can predict household risk for *Aedes aegypti* infestation. If proven useful as an indicator of risk, PCI could be used to streamline future surveys and program activities and possibly reduce program costs.

Knowledge, attitudes, and practices

The KAP survey was designed around the results of the FGDs and IDIs to create questions based on the local context and language [55]. The KAP will be pilot tested in a neighbouring

community and revised where necessary. Questions are close ended or are categorized by data collectors at the time of response.

KAP surveys will be conducted at the same time as baseline and endline entomology surveys. Only the household head will be asked to respond. The data will be collected by experienced government staff who will be given two days training before implementation. Each team will have a supervisor who can monitor data integrity and quality. All paper forms are submitted to the MC supervisor who performs a final check making sure all questions receive a response and skip patterns are followed correctly.

Community health worker monthly monitoring

The coverage of guppy fish and PPF Sumilarv® 2MR will be assessed by ocular inspection of water containers via entomology surveys and the CHW monthly reporting form as described in the adherence section. Coverage is expressed as percentage of containers with guppy fish or Sumilarv® 2MR of the total households or containers examined.

Location

The geographical location of each village within the trial and all households in the Entomology/KAP surveys will be recorded by a handheld Global Positioning System and plotted using ArcGIS® version 10 (Environmental System Research Institute, California) for spatial analysis and for presentation purposes.

Climate

General climate data (rainfall, temperature and humidity) will be recorded at one of the intervention health centres using a rain gauge and a Hobo onset data logger (all villages have virtually the same climate). Data from the all United States National Aeronautics and Space Administration (NASA) satellites on climate will also be available including air pressure, air temperature, atmospheric moisture, evaporation, precipitation, and wind [56].

Data management

Double data entry into EpiData (EpiData Association, Denmark) is completed by an experienced data entry company. The process of data cleaning is being handled by MC staff. The original forms are kept in a secure locked cabinet in the MC Phnom Penh office, and will be available during data cleaning and analysis. Surveys will be maintained in storage for a period of two years after completion of the study.

Statistical methods

All statistics will be performed in R (Murray Hill, New Jersey) and Stata® (College Station, Texas)

Primary outcome

Adult female *Aedes* density will be summed over follow-up time points to give a single rate per cluster. This will be analysed by negative binomial regression using the number of adults as the response, and the logarithm of the sampling effort (that is, person-time spent aspirating) as an offset. Hence, this analysis will yield density ratios. The primary analysis will not be adjusted, but secondary analysis will include an analysis adjusted for the baseline density.

Secondary outcomes

Secondary outcomes including entomological indices such as HI, CI, BI, PPH, and PPP and dengue viral infectivity rate will also be analysed by the above methods.

Missing data

Missing data will be reported as recommended by Díaz-Ordaz et al. [57] and their impact may be explored in secondary analyses.

Data monitoring

In accordance with the findings of Grant et al., we have not established a Data Safety Monitoring Board for this study as it is not a "clinical trial evaluating a therapy with a mortality or irreversible morbidity endpoint" [58]. However, a Technical Steering Committee (TSC) was established which will meet at least every six months and address any safety or other concerns that may arise. The TSC will have one member from each of the partner organizations including the government and WHO. HC and CHW staff have been advised to contact MC staff should any adverse event be detected through passive monitoring as a result of project activities. CHW monthly monitoring forms will also record any adverse events (such as tingling in the hands after touching PPF or gastrointestinal effects after potential exposure of PPF to the mouth in that are reported. Any event will be promptly reported to the ethics committees and government partners. If an end to the trial is needed, the decision will come from the chair of the TSC. However, no

harms are foreseen, and trials of similar products have not experienced any adverse events or unintended effects [16, 17].

Access to data

All co-principal investigators and partners will be given access to the cleaned data sets without identifiers, which will be stored on the Malaria Consortium SharePoint site and will be password protected. The final dataset will also be stored in the Cambodian National Centre for Parasitology, Entomology, and Malaria Control central repository.

Ancillary and post-trial care

In the event of any harm associated with the protocol Malaria Consortium will be responsible as the trial sponsor. The control group will be receiving routine interventions from CNM as described above and will continue to receive them after the close of the project. After the end of the project the lead institution, Malaria Consortium, will continue to advocate for and encourage uptake of any policy recommendations that come from the study

Dissemination policy

The principal investigator (Jeffrey Hii) will ensure that the results of the trial are published regardless of outcome. At least every six months results will be shared with the Technical Steering Committee. In addition to reporting the results in peer-reviewed journals, the results will be disseminated at the provincial level and national level to all project stakeholders. All documents and study materials will be made available in a tool kit that will be given to all government stakeholders and partners. The investigators will also disseminate their findings in

international scientific conferences. Reporting will follow the guidelines in the CONSORT statement [59]. Authorship will follow Malaria Consortium authorship guidelines, which require substantive contributions to the design, conduct, interpretation, and reporting of a trial. The full protocol, household-level dataset, and statistical code will be placed in the Cambodian Ministry of Health's central repository within six months of completion where all interested researchers can request access.

3.3 Discussion

Due to the rise in dengue cases [3], and the current lack of effective vaccines and therapeutics there is an urgent need to develop more effective vector control methods [22]. These methods together with the development of new vaccines [12], genetic control of mosquitoes [14, 15], and new therapeutic drugs [60] will be essential in reducing dengue prevalence throughout the world. Additionally, evidence suggests that the main vector tool in Cambodia (temephos) is becoming less effective [19, 20], and a need to assess new sustainable vector control methods in this context exists [22].

Recent studies have suggested use of larvivorous fish to be effective in vector control [21, 22], however many were methodologically flawed, and none have used a randomized controlled design [37]. The studies on previous products similar to Sumilarv® 2MR showed positive results [16, 17], however the new product has not been tested externally beyond small ongoing semi-field trials in Thailand (personal communication, 2015, Muney Serit). Evidence from larger trials is essential when trying to understand the true impact of these vector control tools and in making recommendations to government bodies and donors.

The study area is suitable for the current trial as the disease is prevalent in the selected districts, and the province has a history of dengue outbreaks [20]. The study team is also familiar with the area having conducted multiple dengue research projects in the area and have good relationships with the local authorities and communities in the area.

It would be preferable to have a primary outcome directly related to dengue incidence rather than an entomological one. Finding the appropriate metric to measure disease impact is bedevilled by the effect of human movement on patterns of transmission, and the pronounced temporal and spatial heterogeneity in transmission, which will necessitate very large cluster-randomized study designs. We considered passive surveillance for dengue with rapid diagnostic tests in HCs. Although sensitivity among currently available tests was considered acceptable for routine clinical diagnostics [61] it was not considered high enough for seroconversion studies. No studies had used rapid diagnostics to estimate seroprevalence. Therefore, more expensive and labour-intensive efforts were preferable, such as cohort studies or capture-recapture methods (which have their own limitations [62]) to estimate the true number of cases and using a more sensitive diagnostic tool such as RT-PCR. However, due to budget limitations it was not possible to employ them. Additionally, unpublished data from a recent cohort study in the proposed districts suggest that, given similar number of cases during this study timeframe, and the resources available to the current project, there would not be enough statistical power to show an impact of the likely size on case numbers. (personal communication, Agus Rachmat, 2015). Therefore, the endpoint chosen was the density of adult *Aedes* mosquitoes, which are on the causal pathway to disease.

There is always a need to balance potential benefits and harms during a trial. The potential benefits of the trial are substantial, and trials of similar interventions in the past have not experienced any adverse events or unintended effects [16, 17, 21, 22]. Additionally, because of the low acute toxicity of pyriproxyfen it considered extremely safe and is recommended by WHO for use in drinking water [44].

This trial is designed to measure the reduction in adult and juvenile mosquitoes due to these vector control methods, relative to a control. However, one limitation is that the study was powered to detect a statistically significant difference between each arm compared with the control, and not between the intervention arms. This reduces the ability to see the impact of the PPF. A possible source of bias may be not having data collectors blind to the intervention; however, in this case it is unavoidable as data collection teams will be able to see the interventions in the containers which they sample. Contamination (spill over) of COMBI activities from intervention villages could affect our study by increasing knowledge or use of guppy fish in control areas. However, in the previous study it was found that only about 5% of containers had guppies in the control area at the end of the project [22]. Measurements of guppy fish coverage will also be conducted in control villages to identify the extent of any contamination.

Although these data are being collected within one province in Cambodia, it is likely that the result of this trial could be generalizable to areas with similar ecology within the country and in neighbouring countries. Each country or province will have to make their own decision based on individual contexts. For example, unpublished MC studies in Myanmar showed similar size and

types of containers and community practices in two regions, and interest from government officials in introducing guppies to water containers in response to dengue outbreaks (personal communication, Jeffrey Hii, 2015). However, the decision was made to not introduce guppies in the Philippines as the community acceptance was low and the cool climate in higher altitudes was not suitable for guppy survival and reproduction (personal communication, Jeffrey Hii, 2015).

Trial status

At the time of submission of this manuscript the trial had completed the baseline data collections, enrolment of villages, and randomized allocation of the villages to three study arms.

Declarations

Ethical Approval and Consent to participate

Ethical clearance for this trial has been received by the Cambodian National Ethics Committee for Health Research on Oct 9th, 2014 (ethics reference number 0285). Additionally, ethics approval was received from the London School of Hygiene and Tropical Medicine Observational/Interventions Research Ethics Committee (ethics reference number 8812). The ethical review bodies will review the protocol annually with progress reports from the project teams. Any subsequent modifications to the protocol which may significantly impact or change the study including eligibility criteria, outcomes, or analysis will be communicated to partners and ethical bodies and will be documented in further publications.

CHWs will explain the trial and receive informed consent from the head of the household before providing the interventions (Appendix 3.2). The CHWs will receive prior training on how to seek informed consent. Those who are illiterate or otherwise cannot sign their name will be allowed to give their thumb print. All households will have the ability to remove themselves from the study at any point.

Entomology specimens will be stored at NAMRU-2. Data which contains identifying information such as names, will be de-identified by assigning individuals ID numbers. All village and respondent names will be deleted to ensure no identifying information is included. Data from surveys will be entered and stored into a password-protected computer. All qualitative data will be collected in concordance with the guidelines of the Code of Ethics of the American Anthropological Association (AAA) [63].

Consent for publication

All authors have consented to publication of this article.

Availability of supporting data

The final dataset will also be stored in the Cambodian National Centre for Parasitology, Entomology, and Malaria Control central repository. Entomological specimens will be stored at -20 C in NAMRU-2 laboratories should other researchers be interested in accessing them.

Competing interests

None of the authors have any competing interests to declare.

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Author contributions

JH conceived the study and design with JH. JH will manage the implementation of interventions. JH wrote the first draft of the manuscript. DD, AR, VK, LS, BS, CV contributed to the acquisition of data. DP contributed to design of entomology work. NA and JB contributed to study design and statistical analysis sections of the manuscript. MS contributed to the design of the behaviour change and communication aspects of the trial. SL and LR contributed to the overall design of the trial. All authors critically reviewed the manuscript.

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The trial sponsor is Malaria Consortium. Contact: John Hustedt, Senior Technical Officer, Development House, 56-64 Leonard Street, London, United Kingdom EC2A 4LT. Phone: +44 (0)20 7549 0210. Email: j.hustedt@malariaconsortium.org.

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Figure 3. 1: Example of a 200-meter boundary around selected clusters

Figure 3. 2: Flow chart of cluster selection. Selection of clusters in Kampong Cham, Cambodia.

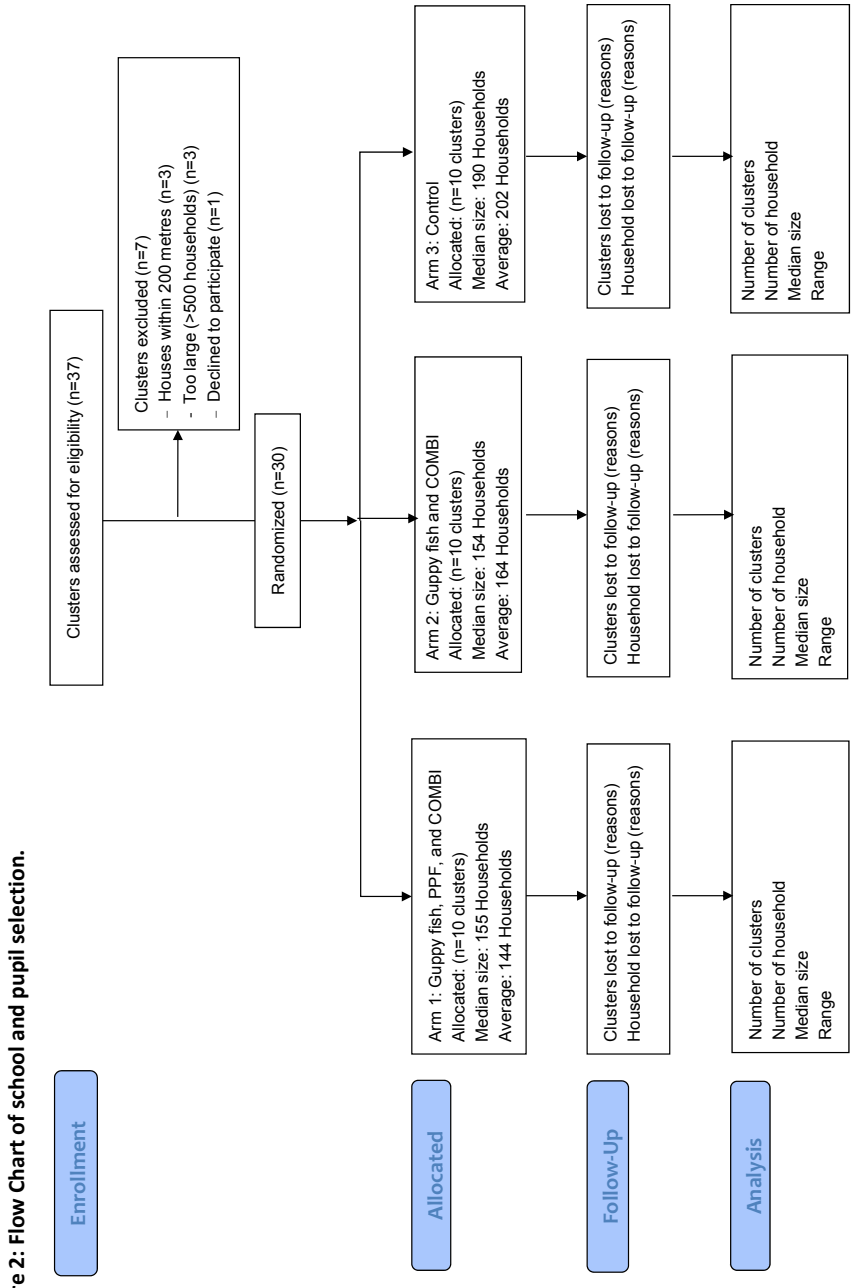
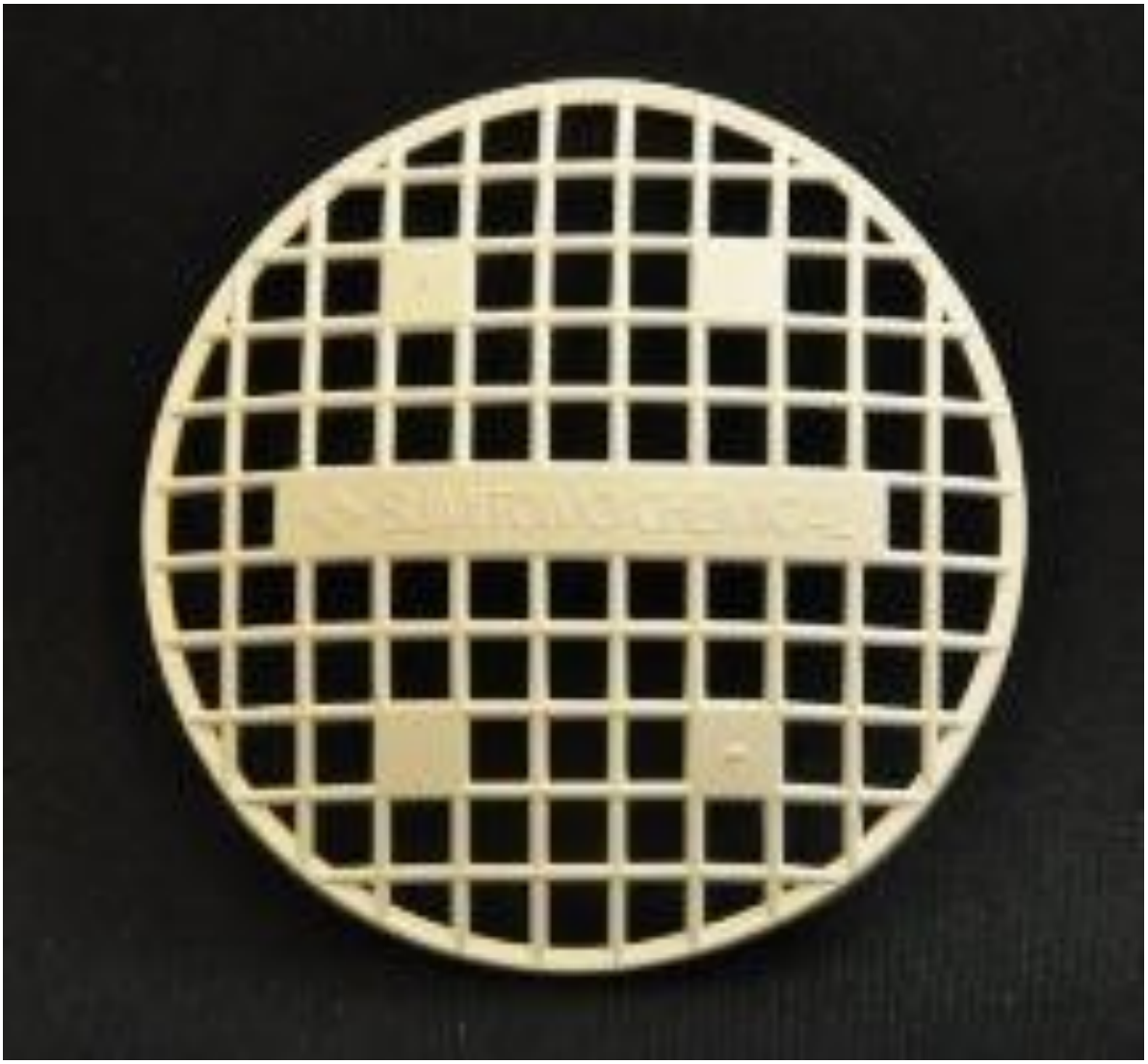


Figure 3. 3: SPIRIT figure with schedule of enrolment, interventions, and assessments

	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			
TIMEPOINT	***Mar 15***	***Mar 15***	***Oct 2015***	***Feb 2016***	***Jun 2016***	***Oct 2016***
ENROLMENT:						
Eligibility screen	X					
Randomization	X					
Informed consent	X					
Allocation		X				
INTERVENTIONS:						
Guppies, Sumilarv® 2MR and COMBI activities, Arm 1			♦—	—	—	—♦
Guppies and COMBI activities, Arm 2			♦—	—	—	—♦
Control, Arm 3			♦—	—	—	—♦
ASSESSMENTS:						
The population density of adult female *Aedes* (primary outcome)			X	X	X	X
Dengue virus infection rate in adult female *Aedes* mosquitoes			X	X	X	X
House index			X	X	X	X
Container index			X	X	X	X
Breteau index			X	X	X	X
Pupae Per House			X	X	X	X
Pupae Per Person			X	X	X	X
Guppy fish coverage			X	X	X	X
Sumilarv® 2MR coverage			X	X	X	X
Percentage of respondents with knowledge about *Aedes* mosquitoes causing dengue			X			X

Figure 3. 4: Sumilarv® 2MR disc (5 cm radius)



Chapter 4: Field efficacy of guppies, pyriproxyfen (Sumilarv® 2MR), and community engagement on dengue vectors in Cambodia: a cluster randomized trial

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

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Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	Lsh1406618	**Title**	Mr
First Name(s)	John Christian		
Surname/Family Name	Hustedt		
Thesis Title	Determining Effectiveness of New Approaches to Dengue Vector Control in Cambodia		
Primary Supervisor	john.hustedt@lshtm.ac.uk		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
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Where is the work intended to be published?	PLOS NTDs
Please list the paper's authors in the intended authorship order:	John Hustedt, Dyna Doum, Vanney Keo, Ly Sokha, BunLeng Sam, Chan Vibol, Neal Alexander, John Bradley, Marco Liverani, Didot Budi Prasetyo, Agus Rachmat, Muhammad Shafique, Sergio Lopes, Leang Rithea, Jeffrey Hii

Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	This PhD is centred on a cluster-randomized trial of which I led the conceptualization, design, and protocol-writing with Jeffrey Hii I was primarily responsible for writing the SOPs, training materials, and field manuals. I was also responsible for all activities of the trial in Cambodia during its execution including supervising all data collection, entry, and management. I also completed all data analysis and wrote reports to the Technical Steering Committee, ethics committees, and donors. I also led the writing of the first draft of the results manuscript. I was a Co-Investigator, and Senior Technical Officer with the Malaria Consortium, for the trial. Members of the PhD Advisory Committee, including my two LSHTM supervisors and Jeffrey Hii — the Principle Investigator of the trial, based in Thailand — provided substantial guidance, including suggested edits to the protocol and other documents.

SECTION E

Student Signature	John Hustedt
Date	July 21, 2019

Supervisor Signature	[redacted]
Date	25 July 2019

Title: Field efficacy of guppies and pyriproxyfen (Sumilarv® 2MR) combined with community engagement on dengue vectors in Cambodia: a randomized controlled trial

Short title: Guppies and pyriproxyfen with community engagement and dengue vectors

John Hustedt[1,2*], Dyna Doum[1], Vanney Keo[1], Ly Sokha[3], BunLeng Sam[3], Chan Vibol[4], Neal Alexander[2], John Bradley[2], Marco Liverani[2], Didot Budi Prasetyo[5], Agus Rachmat[5], Muhammad Shafique[1], Sergio Lopes[1], Leang Rithea[3], Jeffrey Hii[1]

*corresponding author: Tel: +14049695609, E-mail: johnhustedt@lshtm.ac.uk

[1] Malaria Consortium (House # 91, St. 95, Boeung Trabek, Chamkar Morn; PO Box 2116, Phnom Penh 12305, Cambodia)

[2] London School of Hygiene and Tropical Medicine (Keppel Street, London, WC1E 7HT, United Kingdom)

[3] Cambodian National Dengue Control Program (372 Monivong Boulevard, Phnom Penh, 12302)

[4] World Health Organization (No. 177-179 corner Streets Pasteur (51) and 254; Sankat Chak Tomouk

Khan Daun Penh, Phnom Penh, Cambodia)

[5] US Naval Medical Research Unit-2 (#2, St. 289, Boeung Kok 2 commnue, Toul Kork district,, 289 Samdach Penn Nouth, Phnom Penh 1225

Abstract

Background: Evidence on the effectiveness of low-cost, sustainable biological vector control tools for *Aedes* mosquitoes is limited. The purpose of this trial is to estimate the impact of guppy fish, in combination with the use of the larvicide Pyriproxyfen (Sumilarv® 2MR) and Communication for Behavioural Impact (COMBI) activities to reduce entomological indices in Cambodia.

Methodology/Principle Findings: In this cluster randomized controlled trial, 30 clusters comprising of one or more villages each were allocated, in a 1:1:1 ratio, to receive either a) three interventions (guppies, Sumilarv® 2MR, and COMBI activities), b) two interventions (guppies and COMBI activities), or c) control (standard vector control). Entomology surveys among 40 randomly selected households per cluster were carried out quarterly. The primary outcome was the population density of adult female *Aedes* mosquitoes (i.e. number per house) trapped using adult resting collections. Adult female *Aedes* density and mosquito infection rates were aggregated over follow-up time points to give a single rate per cluster. The results from this trial indicate that the interventions resulted in a statistically significant reduction in immature and adult *Aedes* mosquito density when compared to the control. There were no statistical differences identified between intervention arms, although lower guppy coverage in intervention arm two suggests that PPF did help keep mosquito densities low. Data from the KAP and qualitative assessments showed that the interventions were accepted by communities and that they were willing to pay for them.

Conclusions/Significance: The number of *Aedes* females was reduced by roughly half compared to the control in both the guppy and PPF arm (Density Ratio (DR)=0.54 [95% CI 0.34-0.85], p=0.0073), and guppy arm (DR=0.49 [95% CI 0.31-0.77], p=0.0021). The extremely low cost of including guppy rearing in community-based health structures along with the effectiveness demonstrated suggest guppies should be considered as a vector control tool as long as the benefits outweigh any potential environmental concerns. PPF was also highly accepted and preferred over current vector control tools used in Cambodia, however product costs and availability are still unknown.

Trial Registration: Current Controlled Trials ISRCTN85307778; October 25, 2015

Key Words: Dengue, Guppy, Pyriproxyfen, Community Engagement, Vector Control, Cambodia

Author summary

Dengue is one of the most rapidly spreading mosquito-borne viral diseases in the world, is caused by bites of infected *Aedes* mosquitoes, and can sometimes lead to death. Cambodia has one of the highest per-capita incidence rates in Asia. Without a cure or routinely available efficacious vaccine, dengue control relies largely on reduction and avoidance of mosquitoes. In Cambodia, dengue mosquito control activities are focused on larviciding with temephos and pyrethroid based adulticide sprays to which *Aedes* have been shown to be increasingly resistant. This study was designed to evaluate novel biological vector control tools (guppy fish and a controlled release larvicidal matrix) utilizing an integrated vector management approach with community-based methods tailored to the local context. The results indicate that the tools resulted in a statistically significant reduction in immature and adult *Aedes* mosquito density. The interventions were accepted by and communities were willing to pay for them. The results suggest guppies are an ideal vector control tool as long as the benefits outweigh any potential environmental concerns. PPF was also highly accepted and preferred over current vector control tools used in Cambodia, however product costs and availability are still unknown.

4.1 Introduction

Dengue is the most rapidly spreading mosquito-borne viral disease in the world, and is caused by bites of infected *Aedes* mosquitoes, principally *Aedes aegypti* [1]. Dengue is concentrated in the Asian region, which shoulders 70% of the global disease burden. Although a number of promising vaccine candidates are in preclinical and clinical development [2], innovative methods of genetic control of mosquitoes are being developed [3–6], however these interventions are unlikely to eliminate dengue on their own [7]. Therefore, vector control will remain a key component of dengue control in the short and medium term.

In Cambodia, a total of 194,726 dengue cases were reported to the National Dengue Control Program (NDCP) between 1980 and 2008 [8]. However, the real number of cases and cost to society is estimated to be many times higher [9,10]. Previous work showed household water storage jars contained over 80% of *Ae. aegypti* larvae in Cambodia, and these jars became the main target for dengue vector control activities [11].

Since the early 1990s, NDCP has used the larvicide temephos (Abate®) to target large (200-400L) household water containers as the primary means of vector control [12]. This has continued despite tests published in 2001, 2007, and 2018 showing resistance of *Ae.* a*egypti* in several provinces across Cambodia [12–14]. Khun and Manderson (2007) concluded that "continued reliance on temephos creates financial and technical problems, while its inappropriate distribution raises the possibility of larvicide resistance."[12] These problems led researchers to consider alternative control methods including chemical and biological substances (pyriproxyfen (PPF), and *Bacillus thuringiensis israelensis (Bti))* [1,12,15,16], jar covers [11], distribution of

larvivorous copepods and fish [17–19]. The interventions that had the most effective results included the use of larvivorous fish and PPF [1,18].

The use of a larvivorous guppy fish (*Poecilia reticulata*) was evaluated in 14 Cambodian villages [17], and subsequently in a larger study of 28 Cambodian villages [18]. Results from the initial study conducted from 2006-2007 were encouraging as even with low coverage of guppies (in 56% of eligible containers one year after project commencement) there was a 79% reduction in *Aedes* infestation compared to the control area. Despite not having guppies, the smaller or discarded containers in the intervention area had 51% less infestation than those in the control area, suggesting a community-wide protective effect [17]. These results led the WHO and the Asian Development Bank (ADB) to fund a larger scale-up in 2010-2011 which included Communication for Behavioural Impact (COMBI) activities. At the end of the implementation period, an evaluation found that 88% of water jars, tanks, and drums contained guppy fish, suggesting successful establishment of breeding sites. In addition, the Container Index (the percentage of water holding containers infested with *Aedes* larvae or pupae) and the number of indoor resting adult females in the intervention area were near zero, while the control area had a Container Index of 30 [18]. Similarly encouraging results were found in Laos as a part of the same project, although many water containers in the implementation area were too small for guppy survival. This experience indicates that additional tools beyond larvivorous fish are required to target smaller water containers as well as hard-to-reach and cryptic breeding sites.

One potential solution to increase coverage of water containers in the communities is the use of PPF, a juvenile hormone analogue that interferes with the metamorphosis of juvenile *Aedes*

mosquitoes, preventing their development. It can be used in small or contaminated containers unsuitable for larvivorous fish [20]. Studies of the efficacy of PPF in Cambodia showed inhibition of adult emergence (IE) greater than 87% for six months in 2003 [15], and IE above 90% for 20 weeks, and above 80% for 34 weeks in 2007 [1]. A slow-release PPF matrix release formulation (Sumilarv® 2MR) has been developed and shown to be effective in Myanmar [21]. This new product only requires one distribution every six months (the entirety of the rainy season) so reduces operational costs as compared to temephos or *Bti* which have residual efficacy of 2-3 months [16,22].

Yet the efficacy of these measures, like other vector management approaches in the communities, is not only dependent on their entomological efficacy, but requires mobilization and coordination of resources to sustain behaviour changes [23]. In particular, a key challenge for vector control in the communities is how local residents can be involved in and sustain vector breeding source reduction efforts [18]. Recent reviews indicate that a strong communication and behaviour change approach, such as COMBI, has the potential to support vector management programs with very good outcomes [24,25]. For example, two new cluster randomized trials found that educational messages embedded in a community-based vector control approach were effective at reducing *Ae. aegypti* measured through entomological indices [26,27].

Need for a trial

Although there is evidence suggesting the use of guppy fish can be beneficial in dengue vector control, recent reviews show there has never been a cluster randomized trial to evaluate their effect on mosquito indices [28]. This trial has the potential to inform the strategic application of

community-based distribution of Pyriproxyfen and larvivorous fish in an outbreak, during inter-epidemic periods or for broad scale application. This trial will also be the first to our knowledge to evaluate the widescale use of the new Sumilarv® 2MR product in the field. Furthermore, they have never been tested in combination. Therefore, our study is intended to fill the knowledge gaps identified above.

Hypothesis

This trial aims to demonstrate community effectiveness of guppies, PPF, and COMBI activities. The main hypotheses are:

1. Use of guppies, Sumilarv® 2MR and COMBI activities will reduce numbers of *Aedes* mosquitoes, and their infection rates, more than guppies and COMBI alone, or standard vector control activities (such as larval control and information and education material dissemination during outbreaks) as assessed through entomology surveys;
2. COMBI activities will improve the community's knowledge, attitudes, and behaviour related to water use and vector borne disease prevention (such as burning or burying discarded containers, cleaning the environment around the house, and sleeping under a bed net) as assessed through baseline/endline surveys and Focus Group Discussions (FGDs);
3. Guppies and pyriproxyfen will be acceptable among the target villages as assessed by an endline survey and FGDs.

4.2 Methods

Study design and setting

The study is designed as a cluster randomized, controlled trial with three arms. Reporting follows the guidelines in the CONSORT statement [59] (Appendix 4.1). The study has 30 clusters, where each cluster is a village or group of villages with on average 170 households (range 49-405) or 757 individuals (range 250-1769). The rainy season runs from April to November, and the peak dengue season is from May to July. The province of Kampong Cham was selected for its high dengue incidence rate of 1.6 cases per 1000 people in 2014 (personal communication, Hy Ra) and its environmental characteristics similar to most dengue-endemic areas of Cambodia. The clusters were selected based on availability of entomological surveillance data from previous surveys. To minimize potential spill over effects, clusters had to be at least 200 meters from the nearest household outside the cluster since *Ae. aegypti* in this region have an average flight range of 50-100m [29].

Eligibility criteria

Every house within the cluster boundaries was invited to participate in the trial.

Interventions

Selected villages were randomized into one of three study arms (See Table 4.1). Reasons for selecting the interventions for each arm are described above and in more detail in the study protocol [30]. The total trial period for the interventions was 11 months (See Figure 4.1).

Guppies

Two guppy fish (Poecilia reticulata) were placed into each water container greater than 50L in intervention villages (Arm 1 and 2). This is based on larval consumption of guppies determined by Seng et al. [17] and past experiences using guppies in vector control in Cambodia [18]. The guppies were sourced from the original NDCP colony, which was started from guppies found in a rural waterway near Phnom Penh roughly fifteen years earlier. The guppy fish were distributed after the baseline activities through a local community network managed by provincial government authorities [30]. CHWs were provided two jars for rearing. Each month CHWs conducted visual checks and ensured all their assigned households have guppies in all large containers and replaced them if necessary (Appendix 4.2).

Pyriproxyfen matrix release (Sumilarv® 2MR)

The product contains pyriproxyfen incorporated in an ethylen copolymer resin disk, and the PPF is gradually released from the polymer material until it reaches an equilibrium state of the dissolved active ingredient with that in the matrix formulation [31]. Each device is designed to provide coverage for 40 L of water and can be cut into smaller sizes for smaller containers [30]. PPF devices were distributed to containers of size 10-50 litres at the beginning of the trial and replaced after 6 months. Additional devices were left at the HC for CHWs to distribute during their monthly monitoring visit if some were lost or needed to be replaced. The exceptional safety of PPF is reflected in WHO's statements that it is "unlikely to present acute hazard in normal use", "pyriproxyfen does not pose a carcinogenic risk to humans", and PPF "is not genotoxic." As a result of its efficacy, The WHO Pesticide Evaluation Scheme has recommended the use of

pyriproxyfen for mosquito control [32]. Animal models suggest a very favourable mammalian toxicity profile, and extremely low risk for humans using this product [30].

Communication for behavioural impact activities

A rapid formative assessment consisting of FGDs and In-Depth Interviews (IDIs) regarding knowledge, attitudes, and behaviours of community members was completed. The results formed the basis of well-informed COMBI interventions and were used in a message and material development workshop held with key community and district stakeholders [30]. A two-day training was given to CHWs on communication and facilitation skills, roles and responsibilities, and community participation following which they took the lead role in conducting health education sessions twice every month in their community [30]. Monthly meetings were also conducted with CHWs to assess progress, address issues and challenges, and provide them continuous training.

Adherence

In order to improve adherence to the intervention protocols, CHWs performed monthly monitoring checks on each household within the intervention arms, and entomology surveys recorded the presence or absence of each intervention in containers [30]. Project staff also randomly visited CHWs and intervention households to confirm the reliability of data provided.

Primary outcome measures

The primary outcome measure is the population density (i.e. number of mosquitoes per unit of time spent aspirating) of adult female *Aedes* trapped using adult resting collections.

Secondary outcome measures

The secondary outcomes for the trial include:

1. Dengue virus infection rate in adult female *Aedes* mosquitoes
2. House index (HI): Proportion of houses surveyed positive for *Aedes* larvae and/or pupae in any water container
3. Container index (CI): Proportion of surveyed containers containing *Aedes* larvae and/or pupae
4. Breteau index (BI): Number of containers positive for *Aedes* larvae and/or pupae per 100 houses surveyed
5. Pupae Per House (PPH): Number of *Aedes* pupae per household
6. Pupae Per Person (PPP): Number of *Aedes* pupae per person
7. Guppy fish coverage: proportion of eligible water containers with ≥1 guppy fish
8. Sumilarv® 2MR coverage: proportion of eligible water containers with ≥1 MR resin disc
9. Percentage of respondents with knowledge about *Aedes* mosquitoes causing dengue

Sample size

The guppy fish and pyriproxyfen interventions were assessed by four entomology surveys. A sample size of 10 clusters per arm and 40 HHs per cluster for the survey was devised using the Hemming and Marsh method [33] assuming a mean of 0.1 adult female resting *Aedes* per household in the intervention arms compared to 0.25 in the control arm for each collection based on previous studies. The households were randomly selected each collection. The intra cluster correlation (ICC) was assumed to be 0.01 based on previous studies [30]. Additionally, a sensitivity analysis was conducted up to the median value of ICCs for outcome variables (0.03)

as found by an analysis conducted by Campbell et al. [34]. Our analysis determined that ICC values between 0.01 to 0.03 would have 91 to 75% power, respectively.

The impact of COMBI activities in the communities was evaluated through Knowledge, Attitudes, and Practice (KAP) surveys. A sample size of 10 clusters per arm and 20 HHs per cluster was devised, again using the Hemming and Marsh method [33], assuming a 22.5% change in KAP indicators from 40% to 62.5% in intervention villages and no change in the control villages over the course of one year [30].

Allocation

Clusters were randomly assigned with a 1:1:1 allocation through a public randomization process. Village chiefs from all clusters and HC chiefs from all HCs were invited to a central point along with local and national authorities, where allocation took place. Allocation concealment was accomplished by having each cluster representative choose one folded up paper with a printed label referring to arm one, two, or three.

Data collection methods

Data were collected at 0, 4, 8, 12 months post-intervention, unless otherwise mentioned. The timing was also meant to capture data over different season (e.g. heavy rain, light rain, and dry seasons). The project employed the following methods:

Entomology

A baseline survey was conducted prior to start of interventions. An endline survey was conducted one year after the baseline. Two additional surveys during the dry season (4 months post intervention) and light rain (8 months post intervention - peak dengue season) were also conducted. The survey methodology was developed following the WHO guidelines for entomological collections [35] and detailed in the study protocol [30]. The survey team also completed a rapid assessment tool (Premise Condition Index) (PCI) [36] to identify whether the scores can predict household risk for *Ae. aegypti* infestation (Appendix 4.3).

Knowledge, attitudes, and practices

KAP surveys were conducted at the same time as baseline and endline entomology surveys. Details on the methods can be found in the study protocol [30]. The secondary outcome measure included was whether participants knew dengue is transmitted by *Aedes* mosquitoes; however, it should be noted that the word *Aedes* when translated into the local language (Khmer) is "kala" which means feline and is most often interpreted as tiger (Appendix 4.4).

Community health worker monthly monitoring

The coverage of guppy fish and PPF Sumilarv® 2MR were assessed by ocular inspection of water containers via entomology surveys and the CHW monthly reporting form as described in the adherence section. Coverage is expressed as percentage of containers with at least two guppy fish or one Sumilarv® 2MR of the total households or containers examined.

Climate

General climate data (rainfall, temperature and humidity) were recorded at one of the intervention health centres using a rain gauge and a Hobo onset data logger (all villages have virtually the same climate).

Data management

The first two entomology surveys and the first KAP survey were recorded on paper, and double data entry was performed using EpiData (EpiData Association, Denmark) by an experienced data processing company. Due to factors including budget, timeliness, and need for data cleaning, the subsequent two entomology surveys and final KAP survey were recorded electronically on Samsung tablets (Samsung Group, South Korea) and uploaded to ONA servers (ONA, USA).

Mosquito Testing

Adults female *Aedes* mosquitoes were pooled together by cluster with a maximum of 10 per pool, and an expected minimum infection rate of 3-7% based on other studies [37,38] . Flavivirus detection in adult female mosquitoes followed the protocol set out by Pierre et al. [39] using a set of universal oligonucleotide primers. Samples identified as positive for flavivirus were then put into a rapid assay for detecting and typing dengue viruses [40]. All pools had positive and negative controls to ensure the tests were working properly.

Statistical methods

All statistical analyses were performed in R version 3.5.0 (Murray Hill, New Jersey) and Stata® version 14.2 (College Station, Texas).

Primary outcome

Adult female *Aedes* density was summed over follow-up time points to give a single rate per cluster. This was analysed by negative binomial regression using the number of adults as the response, and the logarithm of the sampling effort (that is, person-time spent aspirating) as an offset. Hence, this analysis yielded density ratios.

Secondary outcomes

Secondary outcomes including entomological indices such as BI, PPH, and PPP were also analysed by the above methods. The indicators which were proportions (CI and HI) were analysed using binomial regression.

Data monitoring

In accordance with the recommendations of Grant et al., we did not establish a Data Safety Monitoring Board for this study as it is not a "clinical trial evaluating a therapy with a mortality or irreversible morbidity endpoint" [41]. However, a Technical Steering Committee (TSC) was established which met at least every six months and addressed any concerns that arose [30]. Additionally, participants were told to report any adverse events directly to project staff or CHWs and seek medical attention immediately. CHW monthly monitoring forms include a line to report any adverse events that have taken place. Any report of harm or adverse events was reported directly to the TSC.

Access to data

All co-principal investigators and partners were given access to the cleaned data sets without identifiers, which were stored on the Malaria Consortium SharePoint site and were password protected. The final anonymized dataset is attached as supporting material and will also be stored in the Cambodian National Centre for Parasitology, Entomology, and Malaria Control central repository. Entomological specimens are stored for two years at Malaria Consortium offices should other researchers be interested in accessing them.

Ethical approval and consent to participate

Ethical clearance for this trial was received by the Cambodian National Ethics Committee for Health Research on Oct 9th, 2014 (ethics reference number 0285). Additionally, ethics approval was received from the London School of Hygiene and Tropical Medicine Observational / Interventions Research Ethics Committee (ethics reference number 8812). CHWs explained the trial and received informed consent from the head of the household before providing the interventions [30]. Those who were illiterate or otherwise could not sign their name were given the option of giving their thumb print. All village and respondent names were deleted to ensure no identifying information was included. Data from surveys were stored in a password-protected computer. All qualitative data were collected in concordance with the guidelines of the Code of Ethics of the American Anthropological Association (AAA) [42].

4.3 Results

Baseline results

In the baseline results the control arm had a slightly larger number of houses/people than in

intervention arms (Table 4.2). The sex and age distribution of household heads was similar between the three arms. The mean number of containers, positive containers, BI, and PPP at cluster level were all larger in the guppy only arm (arm 2) than others, while the mean number of adult *Aedes* females per cluster was similar between arms.

Primary outcome

Over the intervention period, the population density of adult female *Aedes* was significantly less in both the guppy + PPF arm (Arm 1) (Density Ratio (DR)=0.54 [95% CI 0.34-0.85], p=0.0073), and guppy arm (Arm 2) (DR=0.49 [95% CI 0.31-0.77], p=0.0021) relative to control (Arm 3). However, the difference between the two intervention arms was not significant (DR=1.10 [95% CI 0.69-1.74], p=0.6901) (Table 4.3). The mean number of adult *Aedes* females was the highest in the light rain season and lowest in the rainy season. (Figure 4.2).

Secondary outcomes

None of the mosquito pools tested were positive for dengue virus; consequently, the minimum infection rate was 0%. The most commonly used entomological indexes (BI and PPP) are reported here, where correlated indices (CI, HI, and PPH) are listed in the supplementary tables (Table S4.1).

Breteau index

Over the intervention period, the BI was significantly less in both the guppy + PPF arm (Arm 1) (DR=0.65 [95% CI 0.50-0.85], p=0.0016), and guppy arm (Arm 2) (DR=0.63 [95% CI 0.48-0.82], p=0.0006) relative to control (Arm 3). The difference between the two intervention arms

was not significant (DR=0.97 [95% CI 0.73-1.27], p=0.7982) (Table 4.4). The biggest difference between arms was seen during the dry and light rain or rainy seasons (Figure 4.3).

Pupae per person

Baseline results show significantly higher PPP in the guppy arm (Arm 2) than the other arms (Figure 4.4). Over the intervention period, the PPP was significantly less in both the guppy + PPF arm (Arm 1) (DR=0.56 [95% CI 0.35-0.91], p=0.0193), and guppy arm (Arm 2) (DR=0.52 [95% CI 0.32-0.84], p=0.0075) relative to control (Arm 3). The difference between the two intervention arms was not significant (DR=0.92 [95% CI 0.60-1.49], p=0.7385) (Table 4.4).

Knowledge, attitudes, and practice survey

The secondary outcome related to the KAP survey is reported here, while the full data set from the KAP survey is in the supplementary files. High levels of knowledge that dengue is transmitted by *Aedes* mosquitoes were reported at baseline among all arms (95.5-98%). Endline surveys showed 100% of participants with this knowledge. Ratios of increased knowledge between baseline and endline were not significantly different between arms with the guppy + PPF arm (Arm 1) (RR=0.99 [95% CI 0.86-0.1.14], p=0.915), and guppy arm (Arm 2) (RR=1.01 [95% CI 0.87-1.16], p=0.943) relative to control (Arm 3) (Table 4.4).

Coverage of guppy fish and Sumilarv® 2MR

Coverage of guppy fish (proportion of eligible water containers with ≥1 guppy fish) before replacement in Arm 2 rose to nearly 80% after one month and stayed close to 70% for most of the intervention period (Figure 4.5). However, in Arm 1 PPF coverage (proportion of eligible

water containers with ≥1 Sumilarv® MR) rose to 80% after two months and stayed high until dropping in March, after which continued health education messages increased coverage back to near 70-80%. Guppy coverage in Arm 1 was notably lower (near 50%) until guppy use was emphasized in March, after which it increased dramatically and then dropped off back to around 50%.

Climate

The average maximum daily temperature in the shade decreased from 34.4° C in the dry season to 31.3° C in the light rain season. The average relative daily humidity and monthly rainfall increased from 60.0% and 10.7 millimetres to 75.2% and 139 millimetres from the dry to light rain season, respectively (Figure 4.6). The rainy season saw much larger amounts of rainfall (near 300 millimetres) than all other seasons.

Adverse events

No adverse events, harms, or unintended effects were recorded during the trial.

4.4 Discussion

Guppies, whether or not in combination with PPF, were able to decrease the number of *Aedes* females (DR=0.49-0.54) and PPP (DR=0.52-0.56) by roughly half compared to the control and resulted in approximately 35% decrease in the BI (DR=0.63-0.64). All other entomological indices also showed similar and statistically significant reductions in intervention arms as compared to the control. There were no statistical differences identified between the two intervention arms, however it should be noted that the trial was not powered to detect those

differences. Regardless, the lack of difference between the arms could also be due to coverage. Guppy coverage was much lower in intervention Arm 1 than in Arm 2 (54% vs 70%), therefore suggesting the use of PPF may have contributed to keeping entomological indicators similar to those in Arm 2.

Although none of the mosquito pools were found to be positive for dengue virus, all the positive and negative controls performed as expected. Additionally, one recent study used a model to simulate the process of mosquito sampling, pooling, and virus testing and found that mosquito infection rates commonly underestimate the prevalence of arbovirus infection in a population. More specifically, they found that in simulated tests with low virus detection ability virus detection (even among pools with 20-50 mosquitoes) failed in a large number of positive samples and most had minimum infection rates of 0 [43]. This suggests that either 1) the minimum infection rate found in this study was the true rate in the population, 2) there was some degradation of RNA which resulted in untrue rates (despite proper cold chain management), or 3) the amount of virus in the pools was not enough to be detected.

It was observed that adherence to guppies was high (70-80%) and consistent when only one intervention requiring behaviour change (guppies) was assigned. In the intervention arm with guppies and PPF adherence to one intervention was highest when focused health education messages were given on that intervention specifically (e.g. guppy coverage in March was highest when guppy use was emphasized and lowest in December to February when PPF usage was emphasized). Similar dynamics have been found with the use of other vector control tools. A recent review concluded that, when applied as a single intervention temephos was found to be

effective at suppressing entomological indices. However, the same effect was not present when applied in combination with other interventions [44]. This suggests that unfortunately no single vector control intervention may be enough to reach elimination of dengue and using multiple interventions which require behaviour change may reduce individual intervention effectiveness. Some studies have suggested combining imperfect vector control with an imperfect medium-high efficacy vaccine could be more efficacious and cost-effective way to reduce dengue cases [45,46].

The results of the KAP survey showed very high baseline knowledge levels which may have resulted from the high number of cases in the study site and from previous government-led anti-dengue efforts in these areas. The knowledge that dengue is transmitted by *Aedes* mosquitos rose to 100% of respondents by the end of the intervention, however even that was not statistically significant between baseline and endline surveys. Similarly, high levels of knowledge on other dengue topics was found in the baseline survey and reported earlier [47]. Interestingly, self-reported vector control practices did not match observed practices recorded in the surveys, and no correlation was found between knowledge and observed practices either [48]. Therefore, an education campaign regarding dengue prevention in this setting with high knowledge levels is unlikely to have any significant effect on practices unless it is incorporated in a more comprehensive strategy for behavioural change (e.g. use of the COMBI method). In addition, to bridge the knowledge-practice gap, there is a need to create an enabling environment at the household, community and health facility level to follow the required behaviours. For example, the vector control knowledge will not be enough until they have a continuous supply of the

recommended interventions (e.g. guppies, PPF, *Bti*) in order to follow the recommended behaviours.

In the previously reported 12 Focus Group Discussions (FGDs) and nine In-Depth Interviews (IDIs) nearly all participants perceived that the interventions resulted in a reduction in *Aedes* mosquitos (both adults and immatures) and dengue cases (Shafique et al., in press). Participants showed high demand for both interventions (guppies and PPF) and were willing to pay between 100-500 riel (0.03-0.13 USD). In addition, several participants began rearing guppies in their home for their personal use, for the children to play with, and to possibly sell in the market. The presence of larvae in the water despite the use of PPF was a source of concern for some participants, although this was overcome in most cases with proper health education through health volunteers. Interpersonal communication through health volunteers was the most preferred method of transmitting prevention messages. Together the entomological, KAP, and qualitative results suggest that the interventions were efficacious and accepted by the community.

However, there is always a need to balance potential benefits and harms of any intervention. Following the recent Zika outbreaks in 2015-2016, there were two groups of ecologists that noticed public health authorities utilizing non-native larvivorous fish (including guppies) in *Aedes* control [49,50]. Both of these groups wrote opinion pieces that gave three strong messages; 1) the use of larvivorous fish in vector control is not effective, 2) the chances of accidental guppy introduction into local ecosystems are large, and 3) that guppies can easily establish populations and damage these aquatic ecosystems. The first point is contradicted by studies which were available at the time, as well as by the current trial [17,18,28]. However,

regarding the other points, guppies are indeed known to be highly plastic and acclimate to new environments [51]. For example, as far back as 1963 guppies have been highly effective in *Culex* control in highly polluted ground pools and waterways in Bangkok, Yangon, and Taipei [52]. In one study it was postulated that female guppies are capable of routinely establishing new populations in mesocosms, and that over 80% of these populations persist for at least two years [53]. Therefore, the key question is what is the ecological impact of guppies being accidentally released into the environment? Despite the strong statements made in the opinion pieces, the underlying evidence seems to be weaker than implied with most introductions made before proper baseline assessments were completed. Studies have shown some effects of guppies on resident fish densities in lab conditions [54,55] , and nitrogen levels in water [56–58], however the extent of these effects across the ecosystem - especially in areas where introduction and naturalization took place many decades ago (such as Cambodia) - are far from settled. A book on evolutionary ecology of the Trinidadian guppy noted that in regard to the impact of exotic guppies “the literature is scant, and the area ripe for research” [57]. The author also noted that manner in which introduced fish species impact native assemblages is incompletely understood, and that issues such as anthropogenic changes to the habitat, such as rise in water temperature, could favour introduced over native species [57].

Measures available to control programs to mitigate the risks of introduction include; 1) restricting breeding sites to areas which can be locked and controlled by the breeders; 2) only distributing fish to key containers in at-risk areas and away from lakes and streams); 3) only distributing male fish to avoid breeding after accidental release by households; or 4) evaluating which indigenous larvivorous fish exist that have similar predation behaviours to guppies and

consider their use. It should be noted that male guppies have been found to consume less larvae than males (123 per day compared to 74 per day) [17], however that consumption rate was more than enough to clear the main breeding jars in Cambodia.

In addition to concerns on accidental release of guppies to the environment, some lab experiments have raised the possibility that putting guppies in containers used for drinking water could increase *Escherichia coli* and other bacteria [59]. However, a recent study (Sidavong et al., submitted manuscript) found the addition guppy fish in Lao and Cambodia made no significant difference to high pre-existing baseline levels of contamination. Therefore, the authors concluded that any contaminating effect may be insignificant when compared with the potential for reducing dengue fever cases and advocated for the inclusion of advice on safe water use to be included in any behaviour change communication programs for guppy introduction.

This study has several limitations. The most important of which is the absence of a primary outcome directly related to dengue incidence rather than an entomological one. Finding the appropriate metric to measure disease impact is bedevilled by the effect of human movement on patterns of transmission, and the pronounced temporal and spatial heterogeneity in transmission, which will necessitate very large cluster-randomized study designs [60,61]. We considered passive surveillance for dengue with rapid diagnostic tests in HCs. Although sensitivity among currently available tests was considered acceptable for routine clinical diagnostics [62] it was not considered high enough for seroconversion studies and no studies were identified that had used rapid diagnostics to estimate seroprevalence. Therefore, more expensive and labour-intensive efforts were preferable, such as cohort studies or capture-recapture methods (which have their

own limitations [63]) to estimate the true number of cases, or using a more sensitive diagnostic tool such as RT-PCR. However, due to budget limitations it was not possible to employ them. Additionally, unpublished data from a recent cohort study in the proposed districts suggest that, given similar number of cases during this study timeframe, and the resources available to the current project, there would not be enough statistical power to show an impact of the likely size on case numbers. (personal communication, Agus Rachmat, 2015). Therefore, the endpoint chosen was the density of adult Aedes mosquitoes, which are on the causal pathway to disease.

Nevertheless, determining the effect of an entomological outcome on dengue transmission is difficult. Multiple studies in Cuba have suggested that a BI of greater than five can be used to predict dengue transmission, although they note that their results can probably not be extrapolated to areas were dengue transmission is endemic [64,65]. A recent study from Peru did show a statistically significant association between 12-month longitudinal data on Aedes aegypti abundance (1.01-1.30) and categorical immature indices (1.21-1.75) on risk ratios dengue virus seroconversion (over six months) [64]. However, even the existence of an association remains less clear across geographies, and what the strength of that association would be in Cambodia (with much higher incidence rates) remains difficult to quantify. These efforts are frustrated by the many intersecting factors which determine dengue infection in communities including the probability of infecting and being infected by a mosquito bite, the duration of infection, treatment-seeking behaviour, the risk of fever, which serotypes are present, acquired immunity in the host, coverage of interventions and background prevalence of dengue infections. The availability of quality data for each of these factors is limited in most tropical countries where the infection rates are highest.

Additional entomological limitations include only having one data collection point in each season, and no measure in the change of parity rate of adult females. The indoor resting collection of *Aedes* adult mosquitoes is subject to many challenges including: (i) individual collector performance & efficiency; (ii) density being time dependent; (iii) and housing conditions, architecture, objects, etc. Another possible source of bias is not having data collectors blind to the intervention; however, in this case it was unavoidable as data collection teams were able to see the fish in the containers which they sample. Additionally, as these data are being collected within one province in Cambodia generalizability could be a concern. However, it is likely that the result of this trial could be generalizable to areas with similar ecology and mosquito densities within the country and in neighbouring countries.

Regardless of which products or methods control programs select, a common limitation of control programs is the lack of resources to reach all at-risk premises or geographical areas, which can make identifying key premises or geographical hotspots important to success. This is particularly true in Cambodia, where funds are limited and mostly focused on procurement rather than operational costs. In a concurrent study reported elsewhere, the authors collected PCI data during each of the entomology surveys. Regression models showed that the density of adult *Aedes* females was positively associated with PCI at the household (ratio of means= 1.16 per point on the PCI scale) and cluster level (ratio of means=1.54) (Hustedt et al, submitted manuscript). However, the number of *Aedes* pupae was negatively associated with PCI at the household level (rate ratio = 0.74) and did not have a statistically significant association at the cluster level. ROC curves suggest the PCI score had “rather low accuracy” (AUC=0.52 and 0.54)

at identifying top-quartile premises in terms of adult female *Aedes* and pupae, respectively. These results suggest that although identification of key households is important, caution is warranted in the programmatic use of PCI in areas of similar geography and mosquito abundance. Future research could focus on confirming these results and testing additional indexes or methods could be devised to better identify key premises or hot spots.

In conclusion, the results from this trial indicate that the interventions resulted in a statistically significant reduction in immature and adult *Aedes* mosquito density when compared to the control. There were no statistical differences identified between intervention arms, although lower guppy coverage in intervention arm two suggests that PPF did help keep mosquito densities low. Data from the KAP and qualitative assessments showed that the interventions were accepted by communities and that they were willing to pay for them. The extremely low cost of including guppy rearing in community-based health structures along with the effectiveness demonstrated here suggests guppies should be considered as a vector control tool as long as the benefits outweigh any potential environmental concerns. PPF was also highly accepted and preferred over current vector control tools used in Cambodia, however product costs and availability are still unknown. The qualitative assessment suggests that a context specific and well-informed COMBI and community engagement by giving an active role to communities is the key to the successful dengue control. Additional studies could be done to confirm these results and explore the effect of the interventions is different ecological conditions.

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Table 4. 1: Interventions randomized to each study arm

Intervention	**Arm 1**	**Arm 2**	**Arm 3**
Guppy Fish in key containers (>50L)	X	X	
COMBI activities	X	X	
Direct PPF application (Sumilarv® 2MR) in smaller containers (10-50 L)	X		

Table 4. 2: Baseline summary measures of containers, houses, and people per cluster

	Control	Guppies	PPF + Guppies
Number of Clusters	10	10	10
Number of houses	2016	1641	1435
Number of people	8475	7542	6700
Number of houses surveyed	400	400	400
Percentage of Male Household Heads (Range)	22 (10-45)	23 (10-32)	20 (10-35)
Median Age of Household Head (Range)	42 (17-78)	42 (18-84)	45 (18-88)
Mean Number of Containers Per Cluster (Range)	154 (121-190)	186 (160-219)	165 (110-213)
Mean Number of Positive Containers Per Cluster* (Range)	24.7 (18-62)	36.5 (18-62)	27.7 (11-69)
Mean Breteau Index Per Cluster (Range)	62 (20-115)	91 (45-155)	69 (28-173)
Mean Pupae Per Person (Range)	0.9 (0.2-2.7)	4.0 (0.2-17.1)	1.1 (0.5-2.3)
Mean Adult *Aedes* Female Density Per Cluster (Range)	10 (1-15)	9 (3-24)	11 (2-20)

*Positive is defined as having either *Aedes* pupae or larvae in the container.

Table 4. 3: Mean population density of adult female *Aedes* trapped using adult resting collections per cluster by arm and survey

	Control	Guppies	Guppies + PPF
Baseline (Range)	10 (1-15)	9 (3-24)	11 (2-20)
Dry Season (Range)	20 (3-49)	11 (3-17)	14 (2-25)
Light Rain (Range)	75 (17-181)	29 (4-71)	35 (12-63)
Heavy Rain (Range)	10 (4-23)	12 (2-25)	8 (1-23)
Total (Range)	35 (3-181)	17 (2-71)	19 (1-63)
Density Ratio (95% CI), p-value*	1 (Ref)	0.49 (0.31-0.77), p=0.0021	0.54 (0.34-0.85), p=0.0073
Density Ratio (95% CI), p-value*	**	1 (Ref)	1.10 (0.69-1.74), p=0.6901

*The ratios do not include the baseline data

**The ratio is not given here as it would be redundant

The trapping time was 10 minutes per house

Table 4. 4: Immature *Aedes* indices per cluster by arm and survey

	Breteau Index		
	Control	Guppies	Guppies + PPF
Baseline (Range)	62 (20-115)	91 (45-155)	69 (28-173)
Dry Season (Range)	88 (18-153)	48 (13-93)	54 (15-93)
Light Rain (Range)	130 (73-188)	81 (40-150)	74 (35-125)
Heavy Rain (Range)	58 (20-150)	51 (15-105)	45 (15-73)
Total (Range)	92 (18-188)	60 (13-150)	58 (15-125)
Density Ratio (95% CI), p-value*	1 (ref)	0.65 (0.50-0.85), p=0.0016	0.63 (0.48-0.82), p=0.0006
Density Ratio (95% CI), p-value*	**	1 (ref)	0.97 (0.73-1.27), p=0.7982
	Pupae Per Person		
	Control	Guppies	Guppies + PPF
Baseline (Range)	0.9 (0.2-2.7)	4.0 (0.2-17.1)	1.1 (0.5-2.3)
Dry Season (Range)	1.0 (0.1-3.3)	0.3 (0-0.9)	0.7 (0-1.7)
Light Rain (Range)	2.2 (0.5-7.0)	1.2 (0.1-3.3)	0.60 (0-1.4)
Heavy Rain (Range)	0.7 (0.1-2.1)	0.6 (0.1-2.9)	0.7 (0-1.8)
Total (Range)	1.3 (0-7.0)	0.7 (0-3.3)	0.7 (0-1.8)
Density Ratio (95% CI), p-value*	1 (ref)	0.56 (0.35-0.91), p=0.0193	0.52 (0.32-0.84), p=0.0075
Density Ratio (95% CI), p-value*	**	1 (ref)	0.92 (0.60-1.49), p=0.7385

*The ratios do not include the baseline data
**The ratio is not given here as it would be redundant

Table S4.1: Remaining secondary outcome tables

	Container Index		
	Control	Guppies	Guppies + PPF
Baseline (Range)	16% (5-38)	20% (8-31)	17% (6-35)
Dry Season (Range)	10% (3-14)	5% (1-10)	6% (2-13)
Light Rain (Range)	21% (10-39)	12% (5-21)	14% (8-27)
Heavy Rain (Range)	10% (3-19)	9% (2-17)	7% (2-14)
Total (Range)	13% (3-39)	8% (1-21)	8% (2-27)
Density Ratio (95% CI), p-value*	1 (ref)	0.61 (0.55-0.67), p<0.001	0.61 (0.55-0.67), p<0.001
Density Ratio (95% CI), p-value*	**	1 (ref)	1.00 (0.90-1.11), p=0.991
	Pupae Per House		
	Control	Guppies	Guppies + PPF
Baseline (Range)	4.2 (1.0-11.6)	17.8 (0.8-69.4)	5.3 (2.4-11.4)
Dry Season (Range)	4.8 (0.5-16.7)	1.6 (0.2-4.9)	3.0 (0.2-7.9)
Light Rain (Range)	9.8 (2.5-32.9)	5.4 (0.3-14.2)	2.6 (0.1-6.0)
Heavy Rain (Range)	3.1 (0.4-8.9)	2.7 (0.6-11.7)	3.1 (0.4-8.1)
Total (Range)	5.9 (0.4-32.9)	3.2 (0.2-14.2)	2.9 (0.1-8.1)
Density Ratio (95% CI), p-value*	1 (ref)	0.55 (0.34-0.88), p=0.0130	0.49 (0.30-0.79), p=0.0032
Density Ratio (95% CI), p-value*	**	1 (ref)	0.89 (0.55-1.44), p=0.642

	Know Dengue is Transmitted by *Aedes* Mosquitoes		
	Control	Guppies	PPF + Guppies
Baseline (%, 95% CI)	98% (96.0-1.00)	95.5% (92.6-98.4)	97.0% (94.6-99.4)
Endline (%, 95% CI)	100% (NA)	100% (NA)	100% (NA)
Ratio (95% CI), p-value	1 (ref)	0.99 (0.86-1.14), p=0.915	1.01 (0.87-1.16), p=0.943

*The ratios do not include the baseline data
**The ratios are not given here as they are redundant

Figure 4.1: Flow chart for enrolment, follow up, and analysis of clusters

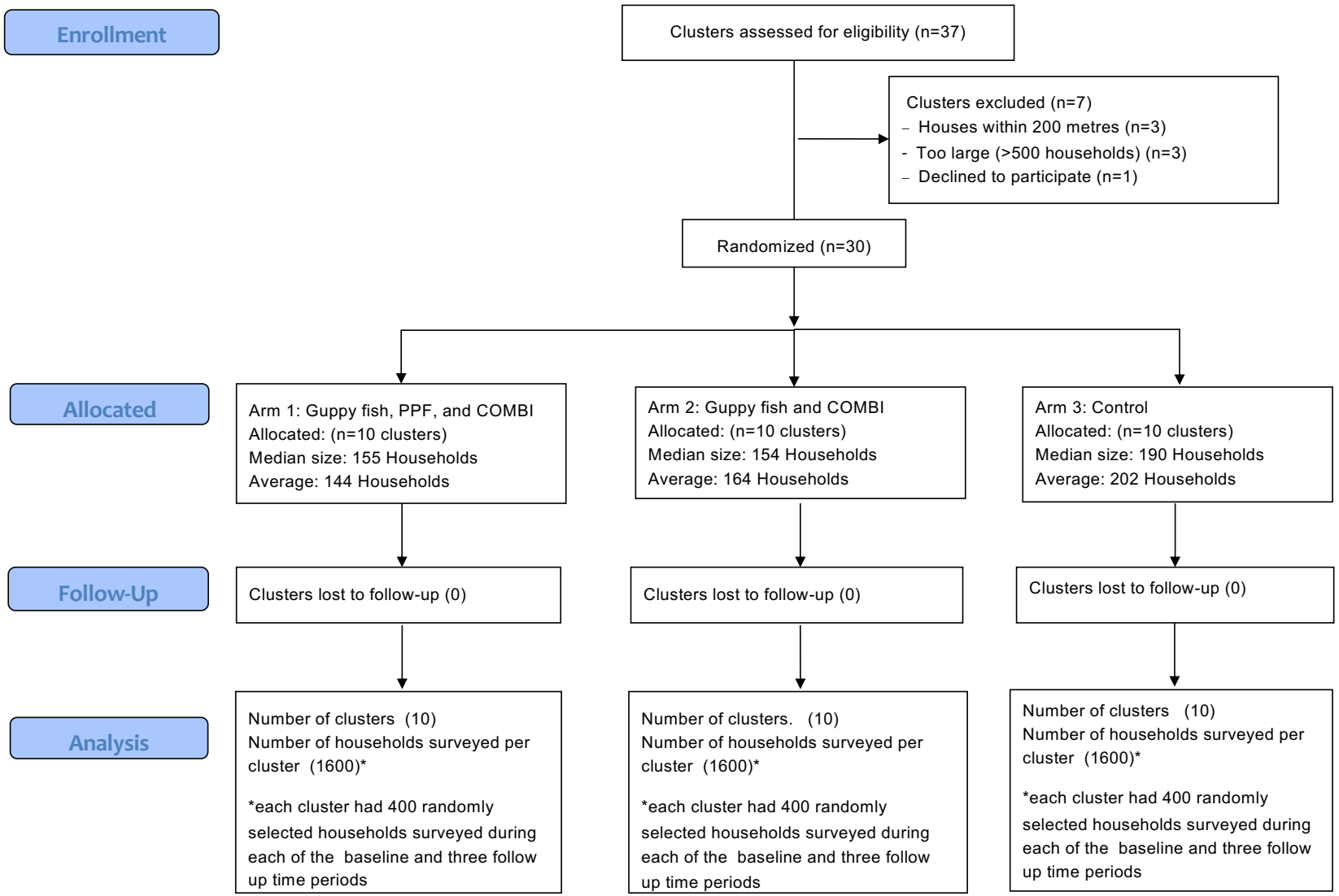


Figure 4. 2: Box plots showing mean number of adult *Aedes* females per household by arm and season, October 2015 – October 2016

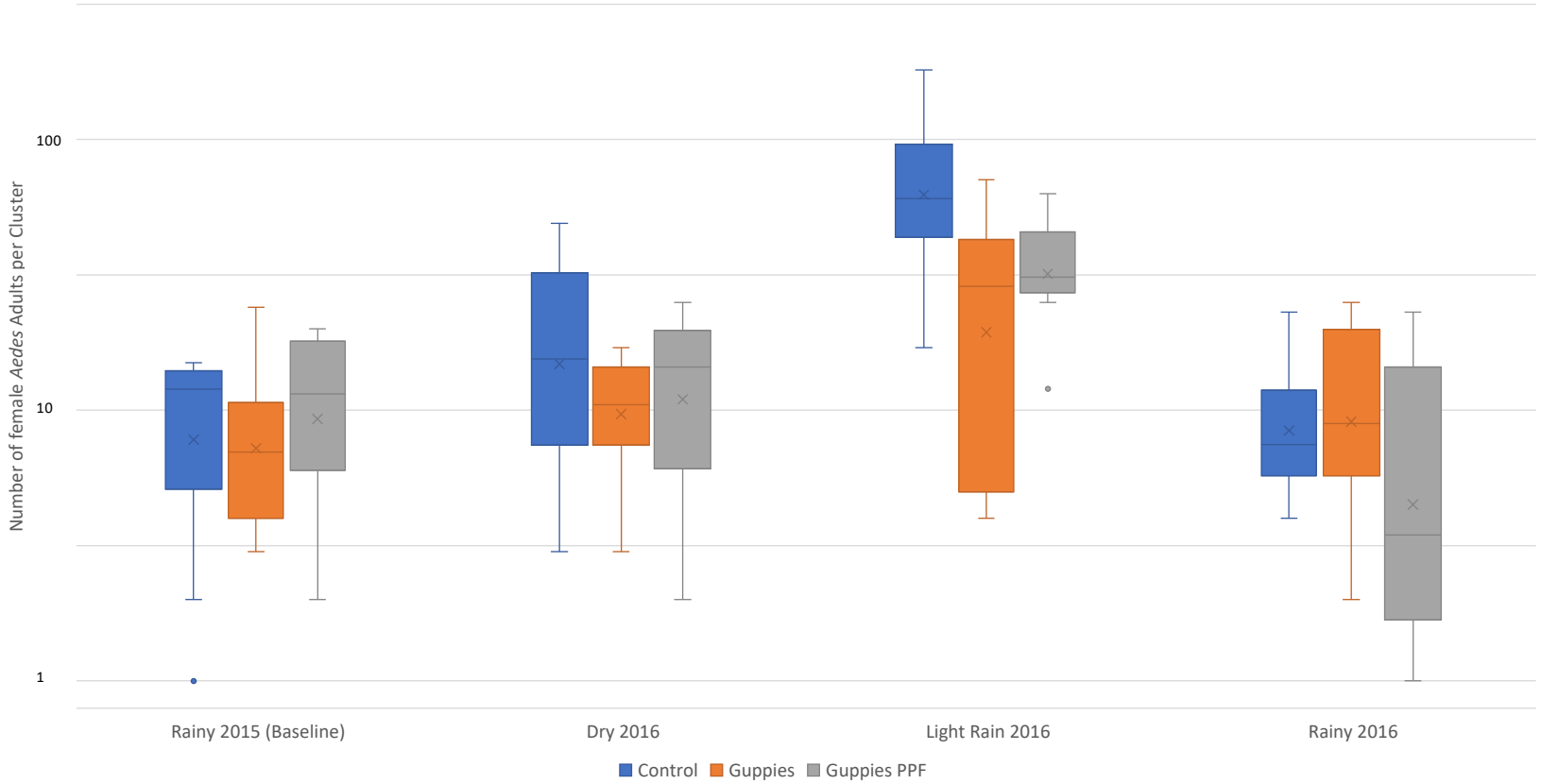


Figure 4. 3: Box plots showing Breteau index by arm and season, October 2015 – October 2016

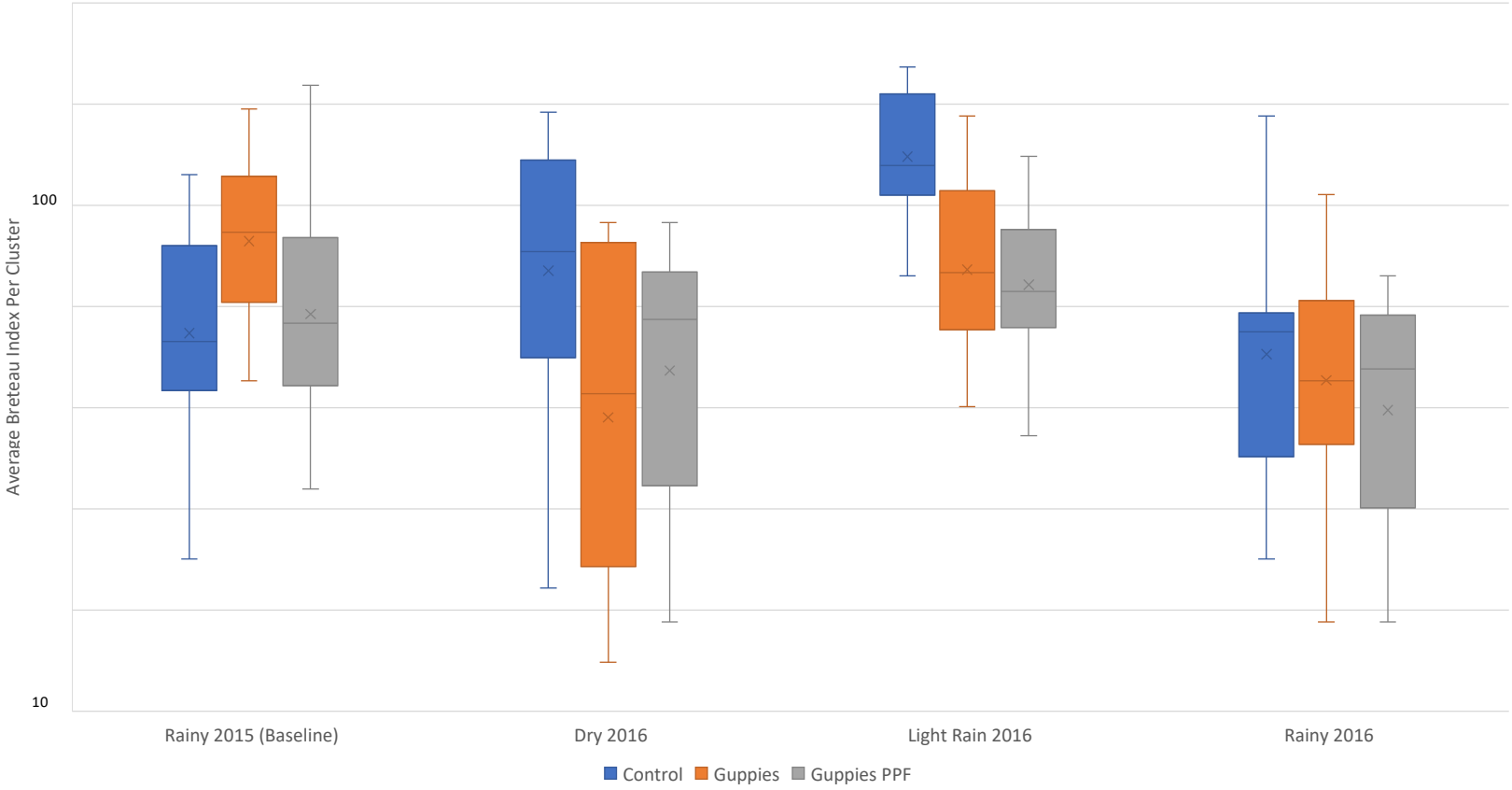


Figure 4. 4: Box plots showing pupae per person by arm and season, October 2015 – October 2016

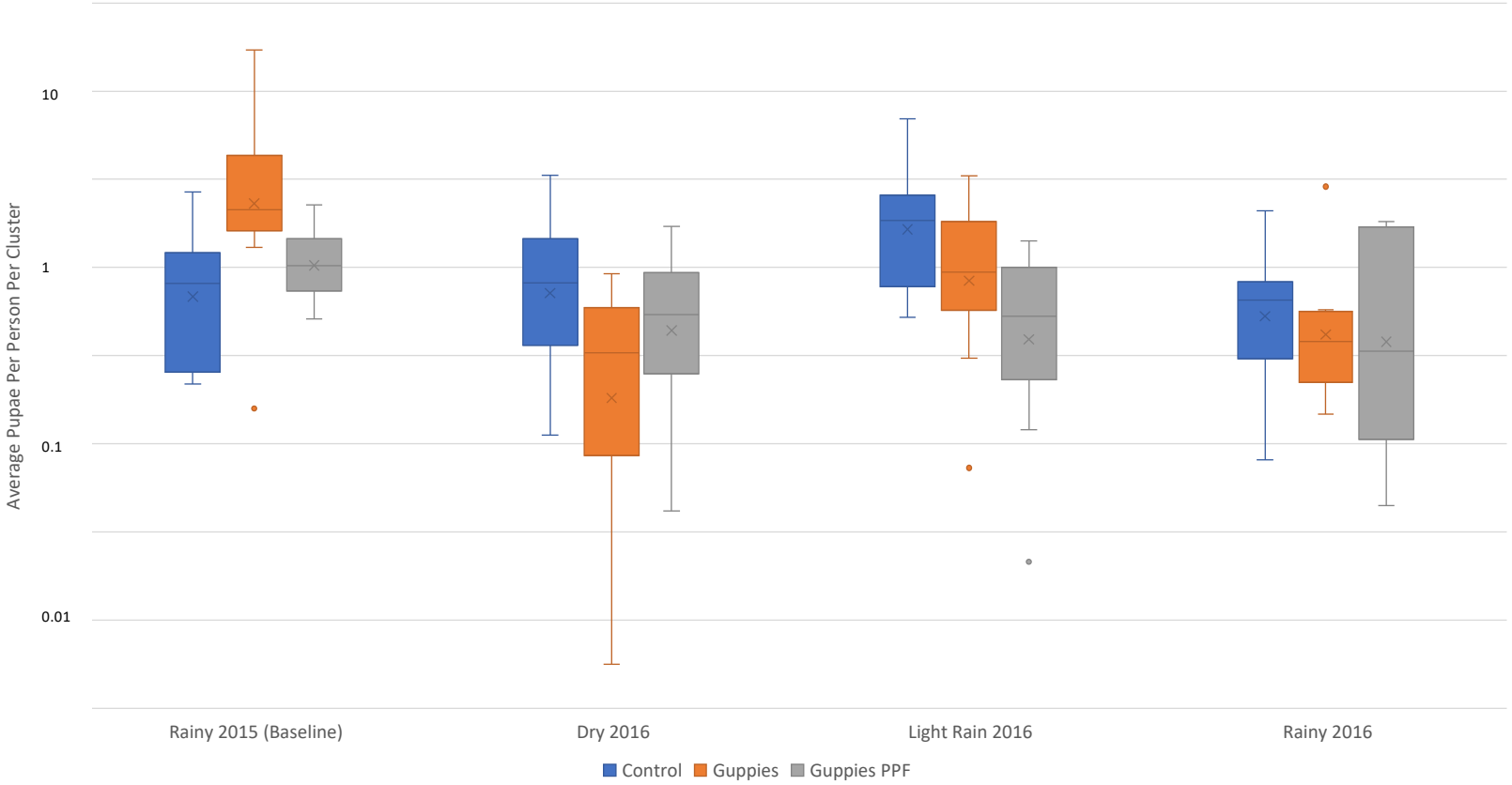


Figure 4. 5: Coverage of guppies and PPF in intervention villages by month, November 2015 - September 2016

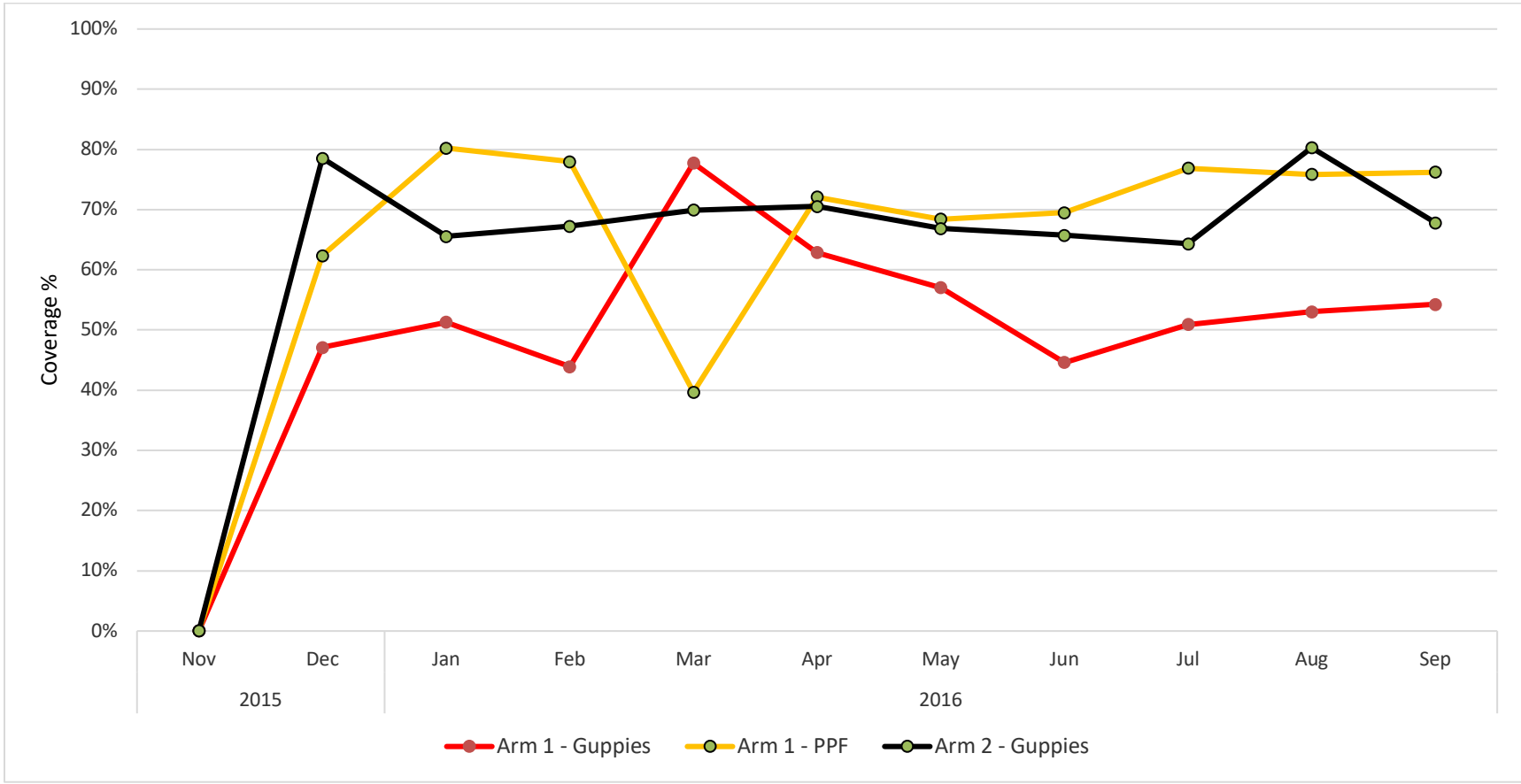
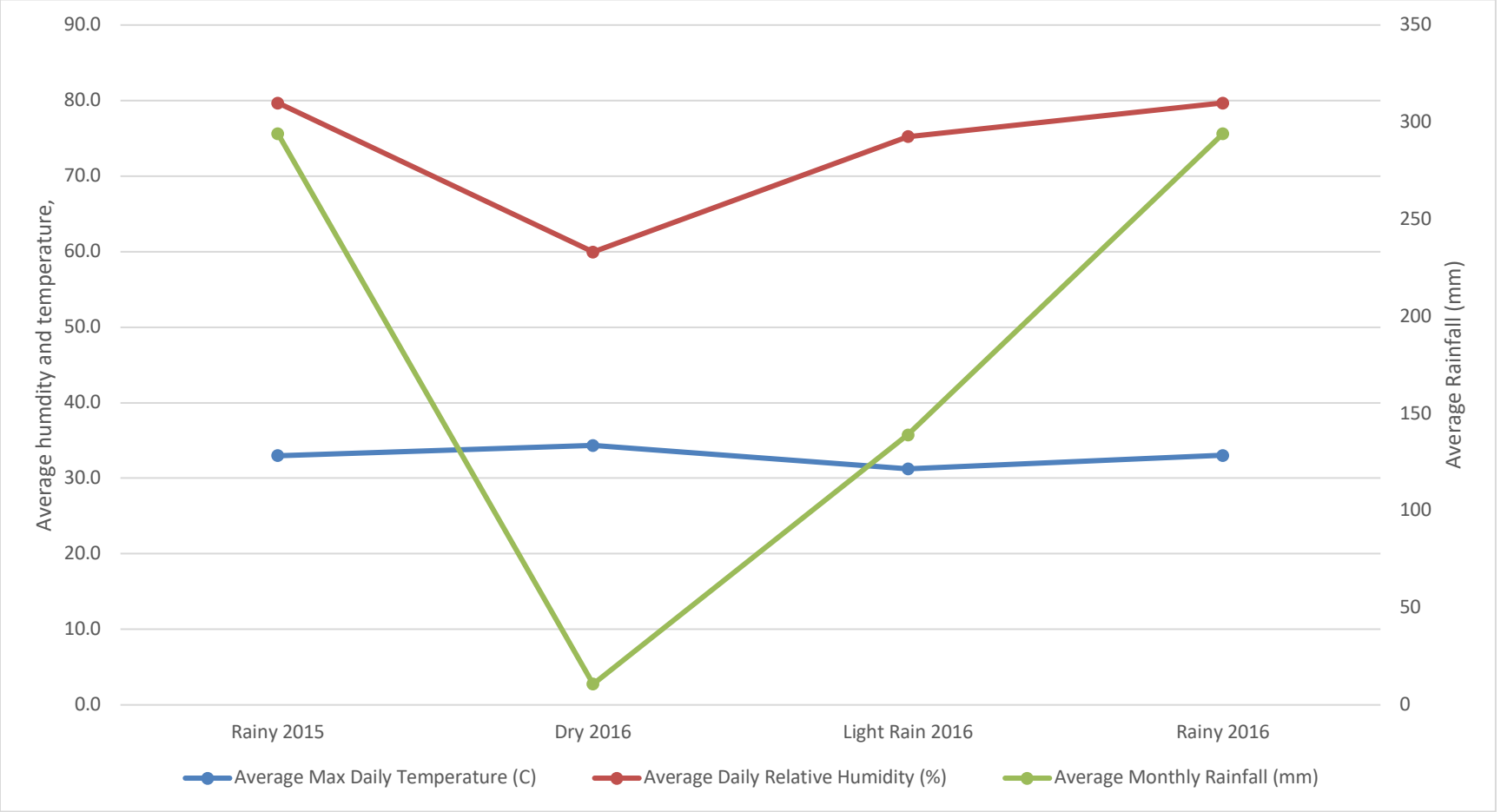


Figure 4. 6: Average maximum daily temperature, relative humidity, and rainfall



Chapter 5: Ability of the Premise Condition Index (PCI) to identify premises with adult and immature Aedes mosquitoes in Kampong Cham, Cambodia

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT

T: +44 (0)20 7299 4646
F: +44 (0)20 7299 4656
www.lshtm.ac.uk

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Student ID Number	Lsh1406618	**Title**	Mr
First Name(s)	John Christian		
Surname/Family Name	Hustedt		
Thesis Title	Determining Effectiveness of New Approaches to Dengue Vector Control in Cambodia		
Primary Supervisor	john.hustedt@lshtm.ac.uk		

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Where is the work intended to be published?	The American Journal of Tropical Medicine and Hygine
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Stage of publication	**Undergoing revision**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceived this study with Jeffrey Hii and led the methodology, data collection, entry, and management, analysis, and writing of the results.

SECTION E

Student Signature	John Hustedt
Date	July 21, 2019

Supervisor Signature	[redacted]
Date	25 July 2019

Ability of the Premise Condition Index to identify premises with adult and immature *Aedes* mosquitoes in Kampong Cham, Cambodia

John Hustedt[1,2*], Dyna Doum[1], Vanney Keo[1], Ly Sokha[3], BunLeng Sam[3], Chan Vibol[4], Sebastien Boyer[5], Marco Liverani[2], Neal Alexander[2], John Bradley[2], Didot Budi Prasetyo[6], Agus Rachmat[6], Sergio Lopes[1], Leang Rithea[3], Jeffrey Hii[1]

[1] Malaria Consortium (Phnom Penh Center, Street Sothearos, Building "H", 1st Floor, Room No. 192, Phnom Penh, Cambodia)

House # 91, St. 95, Boeung Trabek, Chamkar Morn; PO Box 2116, Phnom Penh 12305, Cambodia)

[2] London School of Hygiene and Tropical Medicine (Keppel Street, London, WC1E 7HT, United Kingdom)

[3] Cambodian National Dengue Control Program (372 Monivong Boulevard, Phnom Penh, 12302)

[4] World Health Organization (No. 177-179 corner Streets Pasteur (51) and 254; Sankat Chak Tomouk

Khan Daun Penh, Phnom Penh, Cambodia)

[5] Institut Pasteur du Cambodge, (5 Preah Monivong Blvd (93), Phnom Penh, Cambodia)

[6] US Naval Medical Research Unit-2 (#2, St. 289, Boeung Kok 2 commnue, Toul Kork district,, 289 Samdach Penn Nouth, Phnom Penh 12252)

Key Words: Cambodia, *Aedes*, Premise Condition Index, Dengue, Vector Control

Abstract

Aedes-transmitted diseases, especially dengue, are increasing throughout the world and the main preventive methods include vector control and the avoidance of mosquito bites. A simple Premise Condition Index (PCI) categorizing shade, house, and yard conditions was previously developed to help prioritize households or geographical areas where resources are limited. However, evidence about the accuracy of the PCI is mixed. The current study aimed to contribute to a better understanding of the relevance by collecting data from 2,400 premises at four time points over one year in Kampong Cham, Cambodia. Regression models were then used to identify associations between PCI and *Aedes* adult female mosquitoes and pupae. Additionally, Receiver Operating Characteristic (ROC) curves were used to measure the ability of PCI to identify premises in the top quartile of mosquito abundance. The density of adult *Aedes* females was positive associated with PCI at the household (ratio of means= 1.16 per point on the PCI scale) and cluster level (ratio of means=1.54). However, the number of *Aedes* pupae was negatively associated with PCI at the household level (rate ratio = 0.74) and did not have a statistically significant association at the cluster level. ROC curves suggest the PCI score had "rather low accuracy" (AUC=0.52 and 0.54) at identifying top-quartile premises in terms of adult female *Aedes* and pupae, respectively. These results suggest that caution is warranted in the programmatic use of PCI in areas of similar geography and mosquito abundance.

5.1 Introduction

Dengue is the most rapidly spreading mosquito-borne viral disease in the world, and is caused by bites of infected *Aedes* mosquitoes, principally *Aedes* aegypti [1]. Dengue is endemic worldwide, with a high concentration in the Asian region, which shoulders 70% of the global disease burden. Although a number of promising vaccine candidates are in preclinical and clinical development [2], methods of genetic control of mosquitoes are being developed [3,4], and Wolbachia infected mosquitoes show promise [5,6] these interventions are unlikely to eliminate dengue on their own [7]. Therefore, vector control will remain a key component of dengue control in the short and medium term.

One important aspect of vector control is the elimination of the most productive breeding sites [8]. For example, one study in Australia found that one well and one rainwater tank were responsible for 28% of all immature larvae out of 1,349 premises inspected [9]. Similarly, in Cambodia large water jars, drums, and concrete tanks were found to harbour 90% of the pupal biomass [10]. In addition, studies documented that particularly high levels of *Aedes* productivity can be found in "key premises" [11–14], defined as those with three or more positive containers [9]. In Australia, 1.9% of premises accounted for 47.2% of positive containers [9]. In Ecuador, 11% of households contained 81.7% of pupae during the rainy season and 5% of households contained 80% of pupae during the dry season [12]. Thus, it is clear that the identification of key premises is crucial to inform vector control operations – an activity which can be conducted through pupal/demographic surveys of household water containers. However, the ubiquity of water containers tends to make pupal/demographic surveys laborious [15]. Therefore, additional methods have been explored to identify key premises

without needing to do extensive pupal/demographic surveys, or enter premises, because owners refusing access to premises has been reported as a key challenge [16]. The Premise Condition Index (PCI) is one such approach that could help prioritize outbreak response in terms of *Aedes* infestation risk [9]. This index evaluates the shade, house, and yard conditions of premises to produce risk strata. In addition to targeting treatment of key premises, this method could potentially be used to prioritize villages or other geographical areas when funding or human resources are insufficient to treat all outbreak areas.

Existing evidence of the value of the PCI to inform vector control programs is mixed. The PCI was first described and evaluated in Queensland, Australia, where it was found that inspecting 9.5% of premises with a high PCI score of 8-9 (out of 9) identified 54.4% of infested premises. Comparison of highest to lowest scores indicated a risk of infestation 5.6 times higher, with the number of positive containers 14.3 times higher [9]. Other studies found a association between PCI and the number of positive containers [17–21] and/or positive premises [19,20,22]. PCI has also been used to create risk strata, where a positive correlation ($r=0.968$, $p<0.01$) was identified in Brazil between risk strata and houses positive for *Aedes* albopictus eggs [23]. By contrast, other studies found no association of *Aedes* mosquitoes with PCI [24,25]. Further, serious limitations or missing information exist in many of the past studies. Some studies report associations but do not provide data related to PCI in their paper [18,21,24,26,27] or relied on low sample sizes with wide confidence intervals [19].

Considering these uncertainties, this study aimed to assess whether higher mean densities of adult female *Aedes* mosquitoes and *Aedes* pupae are associated with worse premise conditions,

as measured by PCI; and whether this association leads to reliable predictions of which premises should be targeted for interventions. The study was conducted in Cambodia, a country with one of the highest per-capita incidence rates in Asia, at 0.7–3.0 per 1000 population per year [28,29], and recurring outbreaks every three to five years [30]. The Cambodia National Dengue Control Program (NDCP) developed a protocol to respond to outbreaks, defined as three or more cases in one village per year, which includes applying larvicides (e.g. temephos), adulticides (e.g. thermal fogging with pyrethroids), and distributing information, education, and communication materials. These activities are implemented throughout the entire outbreak villages and can require significant financial and human resources, especially if distances between villages and the number of outbreaks is large. In this setting, if shown to be effective, PCI could potentially be used to prioritize interventions when funds are insufficient to treat all houses or geographic areas. An advantage of the index is that it can be completed quickly and there is no need to enter houses. Although previously published evidence on the relevance of the PCI varies by geography and mosquito life stage, no studies and field evaluations have previously been reported from Cambodia or South-east Asia.

5.2 Materials and Methods

Study setting

The data used in this study were collected during a cluster randomized trial on the effect of guppy fish and pyriproxyfen on entomological outcomes [31], conducted in 30 clusters in two operational districts (ODs) within Kampong Cham province. Each cluster had an average of approximately 200 households or 1000 individuals and included one or more villages that were separated by neighbouring villages by at least 200 meters. Kampong Cham has one of the highest

dengue incidence rates in Cambodia (1.6 cases per 1000 people per year) and the environmental characteristics are similar to most dengue-endemic areas of Cambodia (personal communication, Hai Ra, 2016). The dry season lasts from December to April, the light rain season from April-July, and the heavy rain season from August-October. This study only utilizes data from the pre-intervention baseline surveys and control clusters, which did not receive an intervention, of the aforementioned trial, and are considered to be more representative of the typical conditions in the area. More detailed information about the study site can be found in the trial protocol [31].

Outcomes

The primary outcome was the association between PCI (defined below) and the mean density of adult female *Aedes aegypti* at household level. Secondary outcomes include the association between (1) PCI and the mean density of adult female *Aedes aegypti* at cluster level, and (2) association between PCI and the number of *Aedes* pupae per household and per cluster.

Mosquito collection and PCI scoring

Data were collected at four time points covering all three main seasons: survey 1 was in October/November 2015 during the rainy season, survey 2 was in February/March 2016 during the dry season, survey 3 was in May/June 2016 during the light rain season, and survey 4 was in September/October 2016 in the heavy rain season. The survey methodology was developed following the WHO guidelines for entomological collections [30]. The survey team consisted of experienced government staff who received three days' training before the start of the surveys. All tools and materials were pre-tested during training. Houses within each cluster were selected using a random-number generator applied to the village list managed by the village head.

Larvae and pupae collection were completed using the five-sweep net method [15] for containers larger than 50 litres. For this method, a net of size 20 cm by 33 cm was used. Surveyors turned the net in an anti-clockwise manner five times, then waited 1 min and performed one sweep from the bottom. This method can sample around 35% of larvae and 31% of pupae, and the total number estimated by an adjustment factor [15] (See Table 5.1). For containers of less than 50 L, all the water was poured through the sweep net. All containers within selected households were inspected. All pupae and larvae were put in a plastic bag, labelled, and taken back to the provincial laboratory for identification to the species level for *Aedes*, otherwise to genus.

The adult resting catch was completed using a battery-powered, portable aspirator (Camtech, Phnom Penh, Cambodia) for 10 min per house in the bedrooms and living spaces, starting in the bedroom and aspirating up and down the wall (from floor to 1.5 m) around the home in a clockwise manner. The mosquitoes were kept in a screw-top container inside a cold box and transported to the provincial laboratory for identification to the species level for *Aedes*, otherwise to genus. All adult *Aedes* mosquitoes were sexed. After identification all mosquitoes were taken to the United States Naval Medical Research Unit-2 in Phnom Penh where entomologists confirmed identification of a random sample of 50% of immature and adult mosquitoes.

Each house in the survey was scored on the degree of shade, condition of house, and condition of yard according to the method developed by Tun-Lin et al. [9]. Each category is scored from 1 to 3, and the sum represents the PCI score. The teams were provided with objective measures for scoring in each category (see Table 5.2), a laminated sheet including pictures of example premises for each score and given training to standardize scoring between the three teams. In

addition, a fourth category representing the source of water was scored, however due to the homogeneity of water infrastructure the results are not reported here.

Climate

General climate data (rainfall, temperature and humidity) were recorded at one of the intervention health centres using a rain gauge and a Hobo™ onset data logger (Onset Computer Corporation, Massachusetts, USA) (all villages included in the study have virtually the same climate). Data from the all United States National Aeronautics and Space Administration (NASA) satellites on climate are also available to double check the accuracy of these measurements.

Sample size

Sample size was determined for the needs of the corresponding trial and is discussed in length in the protocol [31]. However, the sample size is at least as large as four other studies which reported a significant association or correlation of PCI with houses or containers with *Aedes* mosquitoes [19–21,32].

Statistical analysis

All analyses were performed in R Studio version 3.5.0 (Murray Hill, NJ, USA) and Stata® version 14.2 (College Station, TX, USA). The association between *Aedes* density and PCI was assessed through negative binomial regression using the number of adults per household as the response and a logarithmic link function. Hence, this analysis yields density ratios as an outcome measure. Models combined data from all seasons and included survey as a fixed effect term.

Additional models including an interaction term of survey and PCI were also run. A likelihood ratio test showed the interaction term to not be statistically significant (p=0.07) and therefore the model with interaction was not included in the results. A similar model was used for the secondary outcomes, with the numbers of pupae, rather than adults, as the response. All models used the robust sandwich estimator of standard errors [33] to account for correlation of responses within clusters.

Associations between PCI and vector density are necessary but not sufficient for PCI to have sufficient sensitivity and specificity to be efficient in practice. Receiver operating characteristic (ROC) curves were used to ascertain the ability of PCI to predict the premises in the top quartile of mosquito biomass. Their accuracy was classified according to the value of the area under the ROC curve (AUC): not informative (AUC≤0.5), rather low accuracy (0.5<AUC AUC≤0.7), accuracies useful for some purposes (0.7<AUC ≤0.9), and rather high accuracy (0.9<AUC)[34].

Ethical approval

Ethical clearance was received by the Cambodian National Ethics Committee for Health Research on 9 October 2014 (ethics reference number 0285). Additionally, ethics approval was received from the London School of Hygiene and Tropical Medicine Observational/Interventions Research Ethics Committee (ethics reference number 8812).

5.3 Results

During the study period a total of 2,400 premises were inspected for the presence of immature and adult *Aedes* and assigned PCI scores. The average monthly rainfall during the study was 11

mm during the dry season (December-April), 139 mm during the light rain season (May-July), and 276 during the heavy rain seasons (August-November). As reported in Table 5.3, the majority of premises (89%) were assigned a PCI score between 5-7, and only 3% and 0.4% were assigned a PCI score of 8 or 9 respectively.

Distribution of adult female *Aedes* mosquitoes by PCI ranking

Table 5.4 shows 26% of houses overall had some adult female *Aedes*, with an average of 0.56 each. The percentage of positive houses and *Aedes* females per house increased during the light rain season, to 58% and 1.88, respectively. The percentage of houses positive for *Aedes* females varied among overall PCI scores (17-33%) and among different seasons (17-58%). The average number of *Aedes* females per house also varied widely among overall PCI scores (0.21-0.73) and over seasons (0.24-1.88). The highest numbers of positive houses and average number of adult female *Aedes* was among premises with PCI scores of 6 and 7.

Table 5.4 shows that 46% of premises and 15% of containers were positive for *Aedes* pupae and/or larvae with an average of seven pupae per house. The proportion of positive premises varied quite widely between PCI scores (22-51%), and between surveys (36-71%) with light rain (peak) season having by far the highest proportion of positive houses (71%). The percent of containers positive for larvae or pupae also varied among PCI scores (7-20%) and surveys (10-21%). Only 1% of premises received a PCI score of three and a few of those premises had extremely high numbers of *Aedes* pupae. The particular reason for the large number of pupae is that two premises had a large water container used for animal husbandry that were not often

cleaned and held hundreds of pupae.

Table 5.5 shows the results of the negative binomial regression models for adult female *Aedes* mosquitoes. The model including two dependent variables (PCI scores and survey) was found to fit best for this analysis. The number of adult *Aedes* females was positively associated with PCI (rate ratio (RR) per point = 1.16, 95% confidence interval (CI): 1.02-1.31). A cluster-level model of adult *Aedes* females by cluster had a slightly higher rate ratio although wider confidence intervals (RR=1.54, 95% CI 1.11-2.08).

Association of immature *Aedes* mosquitoes with PCI

Table 5.5 also shows the results of regression models for *Aedes* pupae. As for adults, the association between the number of *Aedes* pupae and a combination of the three variables was completed. At the house level, the number of pupae were statistically significantly negatively associated with PCI scores (RR=0.74, 95% CI 0.59-0.93). The model investigating the association between number of *Aedes* pupae and PCI/survey at the cluster level was not significant.

ROC curve analysis for predicting the top quartile of adult *Aedes* mosquitoes

The PCI score (at household level) was considered to have "rather low accuracy" predicting premises in the top quartile of adult female *Aedes* mosquitoes, with an AUC of 0.54 (95% CI 0.52-0.56, Figure 5.1). A cut point of 5 had high sensitivity (94%) and low specificity (7%), while 7 had low sensitivity (19%) and high specificity of (83%). For clusters, the PCI score was

also considered to have “rather low accuracy”, with an AUC of 0.64 (95% CI 0.44-0.80, Figure 5.2). No cut points for either curve gives an adequate combination of sensitivity and specificity.

ROC curve analysis for predicting the top quartile of *Aedes* pupae

The PCI score (at the household level) was considered to have “rather low accuracy” when predicting premises in the top quartile for *Aedes* pupae, with an AUC of 0.52 (95% CI 0.50-0.54, Figure 5.3). A cut point of 5 had high sensitivity (93%) and low specificity (7%), while 7 had low sensitivity (16%) and a high specificity of (83%). For clusters, the PCI score was again considered to have “rather low accuracy” when predicting the clusters in the top quartile for *Aedes* pupae, with an AUC of 0.62 (95% CI 0.44-0.80, Figure 5.4). No cut point for either curve gives an adequate combination of sensitivity and specificity. This low degree of accuracy is consistent with the negative association presented earlier.

5.4 Discussion

The PCI was found to be weakly associated with the density of adult female *Aedes* at the household and cluster level, and negatively associated with the number of *Aedes* pupae at the household level. Therefore, our hypothesis that higher mean densities of *Aedes* would be associated with worse premise conditions was correct for adult females, but not for pupae. The five premises with the highest number of *Aedes* pupae (representing 0.1% of total premises) had 25% of the total pupae and had relatively lower PCI scores (one house had a PCI of three). This may have been because the most productive containers were large water storage containers for animal husbandry that are not frequently changed or replaced. More wealthy families and those with nicer houses may be more likely to have more farm animals and therefore need these large

water storage drinking containers. In contrast, 30 premises with the most adult female *Aedes* mosquitoes (representing 0.6% of total premises) had 25% of adult females and they tended to have relatively higher PCI scores (none had scores below five). Therefore, the relative impact of one or two households has less weight on the overall measure with the adults than with immatures.

Similar results have been found in other studies and resulted in affirmations of PCI's effectiveness and suggestions on how to incorporate it into national control programs. Similar positive associations in Mexico (OR 1.27, p=0.001) between PCI and *Aedes* larvae resulted in researchers concluding that the PCI can be an adequate estimator of the *Aedes Aegypti* infestation rate [22]. In Brazil, researchers found a positive correlation between PCI and houses positive for *Aedes* eggs ($r=0.97$, $p<.01$), and stated that the results clearly showed the usefulness of the method [23]. They went one step further and suggested "in the case of dengue outbreaks, by having all representative house indices of the region, it will be much easier and less expensive to control the epidemic". Positive correlations between PCI and house positivity for larvae, pupae, and adult *Aedes aegypti* ($p>0.05$) led authors to advocate to the Brazilian Dengue Control Programme the use of PCI to schedule the vector control teams' visits with different frequencies based on PCI scores [17]. In Mexico, a significant positive correlation between average PCI of a location and the house index was found (OR=1.37, p=0.007), and it was noted that in the near future the authors expected to use information derived from PCI to "focalize integrated dengue vector control on houses/city blocks/neighbourhoods/areas with high levels of PCI (6–9)"[20]. These examples show how relatively weak evidence has been used to advocate for PCI's use and integration into national policy.

However, finding statistically significant correlations does not always mean that the variables will be good predictors [35]. In our study, ROC curves showed that PCI had "rather low accuracy" (AUC=0.54 and 0.52 respectively) to predict premises in the top quartile for *Aedes* adult females and pupae. Additional ROC curves measuring the ability of PCI to predict clusters (as opposed to houses) which represent the top quartile of *Aedes* adult females and pupae also found it to have "rather low accuracy" (AUC= 0.64 & 0.62, respectively). This is especially true when using highly variable outcomes such as immature measures. Therefore, control programs may want to use care when interpreting PCI associations in their area.

There are also several limitations of the PCI methodology to consider including that non-residential premises, public areas, vacant lots and construction worksites are often not inspected and ranked. Andrighetti et al. [17] noted that 21% of the premises in their study could not be ranked as they were empty lots or construction sites and harboured 11.6% of larvae, 20.9% of pupae and 20.8% of adults. In our study we did not include vacant lots, schools, monasteries, or other public areas and therefore results may not be representative of those areas. Additionally, the inability of the inspector to inspect or see into rear yards in some study settings may lead to misclassification [19]. One of the key weaknesses that has been widely reported is that the scoring may not be standardized across individuals, teams, or organizations [19]. One potential way to reduce this variability would be to use unmanned aerial vehicles (or drones) to take aerial photographs that could be scored by one individual or team [36]. Another useful way of using PCI is to classify geographical locations where it has been shown useful would be to assign one team to categorize the areas in known dengue hotspots in advance of outbreaks. Then, the scores could be used to try to identify which hot spots or villages to target and to prepare warnings and

awareness when resources are scarce. Nevertheless, it is unknown how use of PCI to prioritize households or geographical areas would be accepted within the communities [25]. Additionally, this would only work if PCI was not variable between seasons and years.

These results may not be generalizable to areas with more variability in housing conditions, different ecological conditions, or different mosquito abundance profiles. Considerable resources need to be invested in ensuring teams have standardized scoring of PCI, the corresponding PCI cut offs are followed correctly, and evaluating the acceptance of individuals or communities who are not prioritized. These resources may be better spent evaluating other methods to target premises or spent generally on *Aedes* control. Future studies could evaluate the use of PCI in other geographical settings, the effectiveness of PCI to identify premises with dengue infection, or the acceptance by the community of PCI's use where it is found to be effective.

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Disclosures regarding real or perceived conflicts of interest

None of the authors have any competing interests to declare

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Table 5. 1: Type of containers, average volume, and multiplication factors from Kampong Cham, Cambodia

Type of Container	% of Total Containers	Average Capacity (litres)	Range (litres)	Multiplication Factor
Cement Basin (CB)	9.28%	613	559 - 667	4.9
Cement Tank (CT)	9.48%	825	666 - 984	4.3
Water Storage Jar (J)	41.62%	393	380 - 405	3
Miscellaneous- Domestic Use (M)	16.71%	27	25 - 30	1
Small Pot (P)	9.98%	35	18 - 53	1
Flower Vase/Pot/Tray (F)	0.94%	22	1 - 2	1
Tyre (T)	0.82%	45	31 - 59	1
Can/Bottle (C)	0.34%	7	1 - 12	1
Drum (D)	2.61%	138	115 - 160	3
Others (O)	8.24%	31	23 - 38	1
TOTAL	100.00%	214	1-984	1 - 4.9

Table 5.2: Measures for scoring the Premise Condition Index

Premise variables	Description	Classification score
P1. House condition	a. Well maintained, eg newly painted or new house	**1**
	b. Moderately well-maintained house	**2**
	c. Not well-maintained house, eg paint peeling, broken items visible, dilapidated old house	**3**
P2. Yard condition	a. Tidy yard, eg no rubbish or trash evident, well-maintained gardens and lawn	**1**
	b. Moderately tidy yard	**2**
	c. Untidy yard, rubbish and trash abundant and the garden or lawn with overgrown grass	**3**
P3. Shade condition	a. Very little or no shade (<25%), eg no major trees or bush	**1**
	b. Some shade (>25% but <50%)	**2**
	c. Plenty of shade, >50%, e.g. large trees evident, layers of shrubs, green house, plastic tarp sheet or overhanging roofs used	**3**
P4. Water supply and storage	a. Piped water supply only	**1**
	b. Well water supply only	**2**
	c. Rain water and/or river water	**3**

Table 5. 3: Adult female *Aedes* indicators by Premise Condition Index ranking over seasons

All Time Points Combined (n=30 clusters)				
PCI Score	Number (%) of Houses	Houses with at least one *Aedes* female (%)	*Aedes* Females	*Aedes* females per house (mean)
3	30 (1)	5 (17)	11	0.37
4	138 (6)	32 (23)	50	0.36
5	623 (26)	133 (21)	224	0.36
6	1178 (49)	329 (28)	791	0.67
7	327 (14)	97 (30)	239	0.73
8	71 (3)	15 (21)	22	0.31
9	9 (0)	3 (33)	3	0.33
missing	24 (1)	4 (17)	5	0.21
Total	2400 (100)	618 (26)	1345	0.56
October 2015 (Heavy Rain Season) - Control at Baseline (n=10 clusters)				
3	3 (1)	0 (0)	0	0.00
4	31 (8)	8 (26)	10	0.32
5	126 (32)	23 (18)	38	0.30
6	183 (46)	25 (14)	33	0.18
7	41 (10)	11 (27)	19	0.46
8	11 (3)	1 (9)	1	0.09
9	0 (0)	0 (0)	0	0.00
missing	5 (1)	0 (0)	0	0.00
Total	400 (0)	68 (17)	101	0.25

February 2016 (Dry Season) (n=10 clusters)				
3	3 (1)	1 (33)	5	1.67
4	14 (4)	4 (29)	7	0.50
5	187 (47)	42 (22)	71	0.38
6	161 (40)	47 (29)	106	0.66
7	23 (6)	6 (26)	7	0.30
8	6 (2)	1 (17)	1	0.17
9	3 (1)	0 (0)	0	0.00
missing	3 (1)	0 (0)	0	0.00
Total	400 (100)	101 (25)	197	0.49
June 2016 (Light Rain Season) (n=10 clusters)				
3	4 (1)	1 (25)	1	0.25
4	32 (8)	10 (31)	22	0.69
5	54 (14)	29 (54)	64	1.19
6	230 (58)	148 (64)	505	2.20
7	78 (20)	42 (54)	160	2.05
8	2 (1)	0 (0)	0	0.00
9	0 (0)	0 (0)	0	0.00
missing	0 (0)	0 (0)	0	0.00
Total	400 (100)	230 (58)	752	1.88
October 2016 (Heavy Rain Season) (n=10 clusters)				
3	4 (1)	1 (25)	1	0.25
4	13 (3)	2 (15)	2	0.15
5	42 (11)	11 (26)	14	0.33
6	280 (70)	50 (18)	68	0.24
7	56 (14)	7 (13)	9	0.16
8	4 (1)	2 (50)	2	0.50
9	1 (0)	0 (0)	0	0.00
missing	0 (0)	0 (0)	0	0.00
Total	400 (100)	73 (18)	96	0.24

Table 5. 4: Immature *Aedes* indicators by Premise Condition Index ranking over seasons

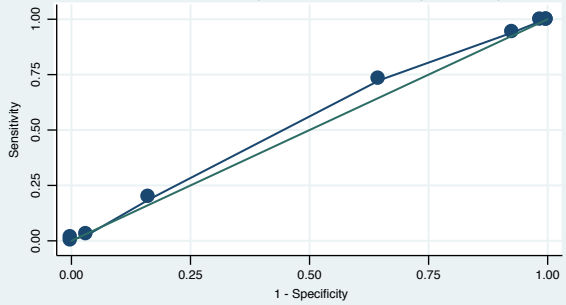
All Time Points Combined (n=30 clusters)						
PCI Score	Number (%) of Houses	Houses Positive (%) for *Aedes* Larvae or Pupae	Container Total	Containers Positive	Number of *Aedes* Pupae	Pupae Per House
3	30 (1)	12 (40)	179 (1)	32	991	33
4	138 (6)	59 (43)	723 (5)	109	887	6
5	623 (26)	250 (40)	3548 (26)	431	5739	9
6	1178 (49)	578 (49)	7016 (52)	1060	8588	7
7	327 (14)	167 (51)	1610 (12)	283	1450	4
8	71 (3)	35 (49)	283 (2)	56	286	4
9	9 (0)	2 (22)	46 (0)	3	9	1
missing	24 (1)	11 (46)	124 (1)	18	49	2
Total	2400 (100)	1102 (46)	13529 (100)	1992	17999	7
October 2015 (Heavy Rain Season) - Control at Baseline (n=10 clusters)						
3	3 (1)	1 (33)	18 (1)	2	11	4
4	31(8)	12 (39)	117 (8)	23	92	3
5	126 (32)	51 (40)	483 (31)	78	594	5
6	183 (46)	72 (39)	726 (47)	105	759	4
7	41 (10)	18 (44)	142 (9)	28	205	5
8	11 (3)	3 (27)	33 (2)	7	3	0
9	0 (0)	0 (0)	0 (0)	0	0	0
missing	5 (1)	4 (80)	25 (2)	4	12	2
Total	400 (100)	161 (40)	1544 (100)	247	1676	4

February 2016 (Dry Season) (n=10 clusters)						
3	3 (1)	1 (33)	35 (1)	10	124	41
4	14 (4)	6 (43)	169 (5)	10	98	7
5	187 (47)	59 (32)	1517 (42)	136	653	3
6	161 (40)	62 (39)	1584 (44)	167	947	6
7	23 (6)	10 (43)	224 (6)	17	81	4
8	6 (2)	3 (50)	46 (1)	8	18	3
9	3 (1)	0 (0)	22 (1)	0	0	0
missing	3 (1)	1 (33)	23 (1)	2	0	0
Total	400 (100)	142 (36)	3620 (100)	350	1921	5
June 2016 (Light Rain Season) (n=10 clusters)						
3	4 (1)	2 (50)	16 (1)	5	6	2
4	32 (8)	20 (63)	152 (6)	33	272	9
5	54 (14)	33 (61)	364 (15)	53	607	11
6	230 (58)	174 (76)	1480 (61)	342	2741	12
7	78 (20)	52 (67)	411 (17)	86	296	4
8	2 (1)	1 (50)	5 (0)	1	0	0
9	0 (0)	0 (0)	0 (0)	0	0	-
missing	0 (0)	0 (0)	0 (0)	0	0	-
Total	400 (100)	282 (71)	2428 (100)	520	3922	10
October 2016 (Heavy Rain Season) (n=10 clusters)						
3	4 (1)	1 (25)	40 (2)	2	32	8
4	13 (3)	7 (54)	99 (4)	15	115	9
5	42 (11)	19 (45)	250 (10)	33	180	4
6	280 (70)	96 (34)	1698 (70)	146	807	3
7	56 (14)	20 (36)	312 (13)	30	98	2
8	4 (1)	4 (100)	26 (1)	7	13	3
9	1 (0)	0 (0)	4 (0)	0	0	0
missing	0 (0)	0 (0)	0 (0)	0	0	-
Total	400 (100)	147 (37)	2429 (100)	233	1245	3

Table 5. 5: Association between each PCI point and the mean density of *Aedes* adult females and pupae at household and cluster level over multiple seasons

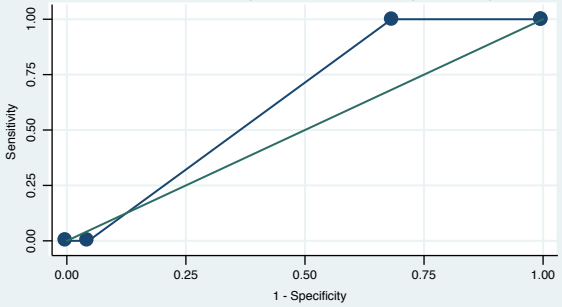
By Household		
	Adult *Aedes*	*Aedes* Pupae
Unadjusted	1.25 (1.11-1.39), p=<0.01	0.74 (0.57-0.96), p=0.02
Adjusted for survey	1.16 (1.02-1.31), p=0.02	0.74 (0.59-0.93), p=0.01
By Cluster		
	Adult *Aedes*	*Aedes* Pupae
Unadjusted	1.80 (1.12-2.88), p=0.01	0.79 (0.32-1.93), p=0.60
Adjusted for survey	1.52 (1.11-2.08), p=0.01	0.78 (0.35-1.73) p=0.55

Figure 1: ROC Curve of PCI and Prediction Values in Predicting the Premises with the Top Quartile of Adult Mosquito Density



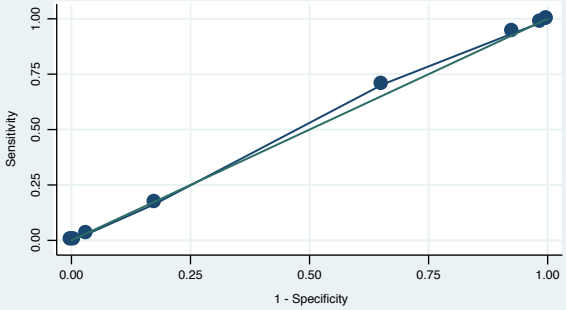
Area under ROC curve = 0.5389

Figure 2: ROC Curve of PCI and Prediction Values in Predicting the Clusters with the Top Quartile of Adult Mosquito Density



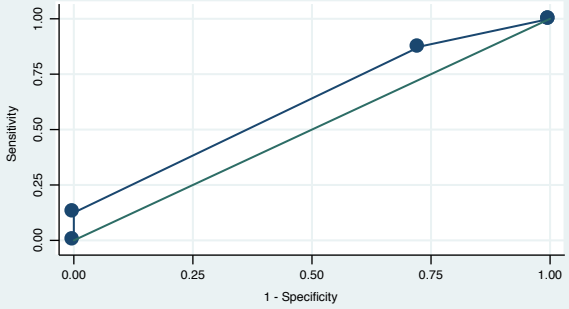
Area under ROC curve = 0.6364

Figure 3: ROC Curve of PCI and Prediction Values in Predicting the Premises with the Top Quartile of Immature Mosquito Density



Area under ROC curve = 0.5160

Figure 4: ROC Curve of PCI and Prediction Values in Predicting the Clusters with the Top Quartile of Immature Mosquito Density



Area under ROC curve = 0.6193

Chapter 6: Discussion

The overall aim of this thesis was to determine the effectiveness of new approaches to dengue vector control in Cambodia. The specific objectives of the PhD are listed below:

1. Perform a systematic literature review on the impact of Pyriproxyfen (PPF) of *Aedes* mosquitoes including to (1) Determine the effect of PPF on a range of endpoints including percentage inhibition of emergence, larval mortality, and resistance ratios; and (2) Determine the different uses, strengths, and limitations of PPF in vector control of *Aedes*.
2. Design a cluster randomized trial in which villages were randomized to either (1) three interventions (guppies, Sumilarv® 2MR, and COMBI activities), (2) two interventions (guppies and COMBI activities), or (3) control (standard vector control).
3. Carry out the trial, analyse the data and report the results of the trial.
4. Determine the ability of the Premise Condition Index (PCI) to identify premises with adult and immature *Aedes* mosquitoes in Cambodia.

This chapter provides further discussion and future directions relating to each of the results chapters. In addition, the chapter provides an overall summary which brings in evidence from other fields such as ecology and discusses public and stakeholder engagement in the material contained in the thesis.

6.1 Systematic literature review of the use of pyriproxyfen in control of *Aedes* mosquitoes

As described in Chapter 2, the results of the review suggest PPF increases *Aedes* larval mortality and Inhibition of Emergence (IE) is strong and consistent. However, the strength of the evidence for different products varies considerably, with PPF granules having highly documented and consistent results and newer products such as PPF dust for auto-dissemination and the use of PPF in Ultra-low Volume sprays, Thermal Fogging, and fumigants showing promise. Many additional novel products have been evaluated (e.g. Sumilarv® 2MR), however evidence for these products is less clear at the moment. The trial described in this thesis will provide some additional evidence for the effectiveness of Sumilarv® 2MR, but it will likely require further testing to determine its true effectiveness in the region.

One additional limitation of the review is that as it was focused on tools that would support the National Dengue Control Program, search terms including other *Aedes*-borne arboviruses were left out. Especially when trying to determine the cost-effectiveness of vector control tools (when disease incidence is known), the inclusion of other *Aedes*-borne diseases could change the conclusion. However, being able to determine a reliable estimate of incidence for other arboviruses in countries like Cambodia where surveillance is challenging (with most of the focus is on dengue) may be difficult.

Future research should focus on these areas where the evidence is less strong such as the development of additional products. Seven of the 19 auto-dissemination studies were published

since 2016, and there has been renewed interest in this method in particular. However, there are several questions still to be answered and obstacles to be overcome. Some of the main research questions still outstanding are (1) the need to develop a basic trap that is affordable for low-income countries, (2) design a method to standardize the application and lethal dose of the PPF dust, (3) determine which surface is optimal, (4) and determine how far apart the devices need to be separated in different ecological environments. Some innovative ideas for new research could include the use of cheap CO_2 emitting devices to enhance numbers of visiting female mosquitoes to dissemination points. In addition to these questions, Sumitomo informed me of their belief that the PPF dust produced in their laboratory is more effective than that produced by pulverizing PPF granules, however that dust is not commercially available, and the potential list price is unknown (personal communication, John Lucas, 2015). In2Care traps do have lots of potential for control of immature and adult stages of invasive container-inhabiting *Aedes* mosquitoes but they are also likely to be priced out of range for most lower income countries like Cambodia.

Additional research is also needed to better clarify the cross-resistance between PPF, temephos, and other insecticide classes to allow control teams to make better informed decisions on which products to recommend and procure for control of *Aedes*-borne diseases. One of the limitations reported is the issue of compliance by the community due to false perceptions by the community that PPF is ineffective as it mainly acts on late instars and people may continue to observe live early instar larvae [1]. Therefore, further studies are required to better understand what communication methods and materials would be most effective to increase community participation in vector control activities. It will also be important to understand the effectiveness of these products in Africa. The majority of studies represented here come from Central/South

America and Asia, with none from India or Africa, despite Africa's significant dengue burden roughly equivalent to that of the Americas (16%) while together Africa and India contribute 50% of dengue cases [2]. It will be important to document the effectiveness of these products in these highly endemic areas [3].

6.2 Trial Protocol

The selection of the primary outcome is one of the most important decisions to make when designing a trial. One of the key limitations of the study was the absence of a primary outcome directly related to dengue incidence rather than an entomological one. This is particularly important given that the World Health Organization (WHO) Vector Control Advisory Group recently noted that modelling based on entomological surrogates is not currently recommended as a replacement for epidemiological RCT data, and should not be used as the primary evidence supporting decisions on the efficacy to public health of new product classes. Malaria Consortium had purchased SD Dengue Duo® RDTs for one of the previous projects and could have used those for the baseline and purchased additional RDTs for follow-up surveys. However, we needed to ascertain the required sample size to determine if we had enough funds to purchase additional tests. It was already known that there was not enough budget for additional lab-based diagnostics. The United States Naval Medical Research Unit-2 provided unpublished data from their previous fever cohort study done in 2011, which was done in many of the same villages as we selected for the study.

Using that data, it was determined that if we assumed that the rate of fever is the same as 2011 rainy season then there would be 672 fever cases per month. If we chose to test all fever cases

during the quarterly entomology survey months, it would require the purchase an additional 2,016 tests. The actual number used may be slightly less since the calculations were based on the rainy (peak) season and some of the surveys would be done during the dry season where the number of cases is expected to be less.

Simultaneously, discussions with Professor Rosanna Peeling from LSHTM and publications on the evaluation of commercially available diagnostic tests [4] suggested that although sensitivity among currently available tests were considered acceptable for routine clinical diagnostics, it may not be considered high enough for seroconversion studies. The study also showed that out of currently available test kits the SD Dengue Duo® had the best performance [4]. In preparation for a potential systematic review on the subject two initial scoping reviews were completed. The first review aimed to determine the reported specificity and sensitivity of Dengue Duo RDTs (Appendix 6.1). A total of 10 studies were then included in the review. The included studies were published between 2012 and 2015. Nine of the studies had original data and one was a meta-analysis. The sensitivity ranged from 58-96% and the specificity ranged from 83-99%. However, four studies did not report what was used as the gold standard for comparison, and the ones who did mention it did not use a standardized method. Therefore, there are limitations to comparing the results across studies. Regardless, the results show that it may be difficult to use RDTs in place of more accurate lab-based diagnostics. The second scoping review focused on determining the use of Dengue Rapid Diagnostic Tests in Sero Surveys (Appendix 6.2). A total of 13 studies were then included in the review and none of the studies used RDTs data as the primary diagnostic in dengue sero-prevalence surveys.

Due to the results of the literature search, and the determination there would likely not be enough funds to purchase the RDTs (or employ nurses needed to properly administer the tests), we decided not to pursue a systematic review or include disease-based endpoints in the study. However, a recently published systematic review on the ability of RDTs to determine dengue serostatus found similar results with four dengue IgG RDTs used in ten studies (with serum used in most of the studies). None of the studies reported RDT data for determining dengue serostatus [5]. The review concluded that modifications to current RDTs are feasible that could optimize the performance of the test in future which could possibly make them feasible for use in seroprevalence studies. Future studies should test this feasibility, which could possibly make doing sero surveys cheaper and easier.

6.3 Trial Results

As discussed in Chapter 4, the results from this trial indicate that the interventions resulted in a reduction in immature and adult *Aedes* mosquito density when compared to the control. There were no statistical differences identified between intervention arms, although lower guppy coverage in intervention arm two suggests that PPF did help keep mosquito densities low. Data from the KAP and qualitative assessments showed that the interventions were accepted by communities and that they were willing to pay for them. PPF was also highly accepted and preferred over current vector control tools used in Cambodia, however product costs and availability are still unknown. There were some technical issues which are covered more fully below.

Ecological Concerns about Introduction of Non-Native Fish

As discussed in Chapter 4, concerns about the ecological effect of introduction of non-native fish used for vector control (including several different species) have been raised by some ecologists [7]. However, following the recent Zika outbreaks in 2015-2016, there were two high profile groups of ecologists that wrote opinion pieces trying to dissuade public health authorities utilizing guppies in *Aedes* control [8,9]. *Science* magazine wrote their own piece on the subject [10]. They contacted me and, following an hour-long interview, wrote a piece on their website which, at the very least, allowed for the perspective of a public health practitioner in the debate (Appendix 6.3).

The topic again arose as Malaria Consortium was fundraising for an expansion of the guppy fish intervention to five high-risk provinces in Cambodia. A potential donor had concerns raised by the aforementioned opinion pieces and was not satisfied and given the lack of available evidence to show the environmental impact of guppies declined to donate to the project.

Due to these experiences, a protocol was developed for a systematic review on the impact on the ecosystem associated with introduction of guppy fish (*Poecilia reticulata*) (Appendix 6.4). I recruited an interdisciplinary team of statisticians, public health practitioners, and ecologists. Hopefully, this review will elucidate what evidence exists or missing and suggest some potential studies that ecologists or public health experts could take on in order to close the knowledge gaps. It may also hold more weight for academically minded scholars and practitioners than writing additional opinion pieces and will allow for evidence-based dialog on the subject.

6.4 The association of the Premise Condition Index with *Aedes* mosquitoes

As discussed in Chapter 5, The Premise Condition Index (PCI) was found to be weakly associated with the density of immature and adult female *Aedes* at the household and cluster level. Similar results have been found in other studies and resulted in affirmations of PCI's effectiveness and suggestions on how to incorporate it into national control programs. However, finding statistically significant correlations does not necessarily mean that the variables will be good predictors [11]. In our study, Receiver Operating Characteristic (ROC) curves showed that PCI had "rather low accuracy" [12] to predict premises in the top quartile for *Aedes* adult females and pupae. Additional ROC curves measuring the ability of PCI to predict clusters (as opposed to houses) which represent the top quartile of *Aedes* adult females and pupae also found it to have "rather low accuracy". Therefore, control programs may want to use care when interpreting PCI associations in their area and assess their ability as predictors before utilizing them in public policy.

ROC curves are also based on cut points, and control programs should be careful in assigning those cut points as it will affect their predictive capacity. In the study presented in this thesis, the cut points were set at the top quartile of premises in terms of *Aedes* immatures or adults, however there are many different theories and suggestions on how to determine cut points [13,14]. Most focus on assessing the value at which sensitivity and specificity are closest to the area under the curve. As discussed earlier, there were a small number of premises which harboured a large number of immature and adult *Aedes,* and this was particularly true with *Aedes* pupae. In our case if you included premises much higher into the top 35-50% you would reach those houses which did not harbour any mosquitoes therefore making those predictions not very useful in

practical terms. Although control programs should try to assess the predictive value of any index, they should also be mindful of selecting useful cut-points when completing any analysis. More research could be done into how to determine the most useful cut points for vector control indexes such as those presented here.

If PCI was found to be useful in predicting and prioritizing vector control activities, there are still limitations that would require further research to improve. One of the limitations of PCI is that scoring may not be standardized across individuals, teams, or organizations [15]. One potential way to reduce this variability would be to use unmanned aerial vehicles (or drones) to take aerial photographs that could be scored by one individual or team. Similar methods have been tried in the past and not found useful, however drones and cameras have improved vastly since this was done twenty years ago [16]. Another way could be to use PCI to classify geographical locations where it has been shown useful and assign one team to categorize the areas in known hotspots in advance of outbreaks. Then, the scores could be used to try to identify which hot spots or villages to prioritize when resources are scarce. Nevertheless, it is unknown how use of PCI to prioritize households or geographical areas would be accepted within the communities [17]. Research would have to be conducted to better understand the best methods for collecting PCI, and how to work with communities so they understand why one area may be prioritized over another. Additionally, this would only work if PCI was not variable between seasons and years.

One important item to note is that the PCI may not have been useful in predicting the density of adult *Aedes* mosquitoes, but it could have been successful in predicting other measures of dengue risk. Risk measures that could be incorporated in the future include the number of infected

Aedes females, the change in parity rate in *Aedes* females, and/or the number of suspected/confirmed dengue cases. The most direct measure of risk would be the dengue incidence rate; however, the accuracy of this measure is complicated by individuals traveling and possibly being infected outside the areas outside those being evaluated. Additional predictors that might be included in PCI are water bodies such as rivers, streams, lakes, large areas of standing water, or specific containers that were found to be higher risk such as large water containers used for animal husbandry.

These results may not be generalizable to areas with more variability in housing conditions, different ecological conditions, or different mosquito abundance profiles. Considerable resources need to be invested in ensuring teams have standardized scoring of PCI, and the corresponding PCI cut-offs are followed correctly. These resources may be better spent evaluating other methods to target premises or spent generally on *Aedes* control. Future studies could also evaluate the use of PCI in other geographical settings, the effectiveness of PCI to identify premises with dengue infection, or the acceptance by the community of PCI's use where it is found to be effective.

6.5 Overall Summary

In summary, the trial presented in Chapters 3 and 4 indicated that the interventions resulted in a reduction of nearly fifty percent in immature and adult *Aedes* mosquito density when compared to the control. As the guppies were found to be effective and locally accepted the recommendation is to consider their use in future vector control activities. Although, the trial itself did not show a statistical difference between intervention arms with and without Sumilarv®

2MR, the results from Chapter 2 suggest PPF (in granule form) have highly documented and consistent results when used in higher doses and distributed every 30-40 weeks. The reason for not detecting a statistical difference between intervention arms could be that it was used only in smaller containers rather than large water containers where the majority of mosquitoes are found in traditional pupal surveys. In addition, the trial was not powered to detect the difference. As Sumilarv® 2MR is not yet registered in Cambodia and the cost is unknown, it is difficult to formulate a recommendation at this time. The results from Chapter 5 suggest that PCI should not be used as a predictor of households with greater *Aedes* densities in this context. Further details on sustainability of the interventions one year after the end of the trial, efforts to use the trial results to draft a National Dengue Strategic Plan, public reactions to the project, and future research directions are discussed in the following sections.

Trial follow-up

In 2016, Break Dengue launched the Community Action Prize which was a competition to reward the best grassroots initiatives that combat dengue in an innovative way. A proposal was developed to go back and assess the community's views about dengue control a year after the project ended and promote the inclusion of community-based vector control (as discussed in Chapter 3 and 4) into the Cambodian National Dengue Strategic Plan for 2017-2020. The proposal was one of two selected out of 67 submitted. Break Dengue also wrote a blog post about the trial discussed in Chapter 4 after the Prize was announced and mentioned how the prize winnings (10,000 Euros) would allow teams to follow up one year after the trial (Appendix 6.5). The prize funds were partially allocated to conduct focus group discussions (FGDs) and interviews with key stakeholders from the previous project.

The results of the FGDs and interviews were encouraging showing that although the number of individuals using guppies in project villages decreased after the project ended, there has been an overall increase with several control villages and those outside the project area beginning to raise the fish. There were also excerpts suggesting that villagers believed that using the fish will stop all dengue infection in villagers, suggesting CHWs should continue to explain that even 100% coverage may not completely stop transmission and villagers are at-risk when they travel outside their villages. Overall the results are quite positive and show that in many villages the larvivorous fish were still being used for vector control in the communities one year after the project has ended. Additionally, together with WHO, Malaria Consortium was able get larvivorous fish introduction included as one potential vector control method in draft version of the National Dengue Strategic Plan for 2017-2020. The final report submitted to the Synergist with some additional results is attached here (Appendix 6.6).

Public reaction to the project

The trial results were presented at scientific conferences (Asian Congress of Paediatric Infectious Diseases (ACPID), American Society of Tropical Medicine and Hygiene Annual Meeting, and the Joint International Tropical Medicine Meeting) and at the project dissemination meeting where journalists were invited. There was also a short film made about the project which was circulated to the media which can be accessed here (https://www.youtube.com/watch?v=vcR7-RCXMMg&t=37s). The presentation at ACPID won the award for best e-poster discussion. Following the dissemination events, Reuters wrote an article that highlighted our preliminary

findings and talked about the need to find cheap, low cost solutions to medical problems (Appendix 6.7).

Local media also wrote reports which highlighted quotes from project staff, WHO officials and the government (Appendix 6.8). Interestingly, they highlighted the reluctance of the government stating that "despite acknowledging that the government's current plan [of using temephos] is more costly than using guppy fish… [they were] not convinced" that the fish were a long-term alternative and did not "know how sustainable it is yet". This was also the sentiment from NDCP government officials when they presented the local dissemination meeting with community health workers in Kampong Cham (i.e. a preferred focus on top-down centrally run interventions). However, the article highlights the positive reactions from all other stakeholders including participants, community health workers, provincial health department officials, and the WHO which said guppy fish are the best solution for Cambodia at the moment. The regional entomologist at WHO said the scale and frequency of dengue outbreaks will fall if deployment of guppy fish was expanded as suggested, which would reduce the dependency on pesticides. In an effort to encourage the government to expand the use of guppy fish the WHO published an article on their website titled "WHO supports targeting *Aedes* mosquito larvae through Integrated Vector Management in Cambodia" (Appendix 6.9). Despite the push from WHO and local stakeholders there still has not been funding received to expand the project or do any further evaluations at a larger scale.

Future research

Following the end of the project, a project dissemination and policy uptake workshop was organized to invite government officials and experts from around the region to hear the results of the project and make recommendations for the way forward. This included working groups that discussed particular topics including prioritization of vector control tools, behavior change communication, case management, and outbreak response. Key stakeholders also met following the workshop where the results of the working groups were discussed and a key summary of recommendations and action points for follow up was agreed. The summary which included future research topics was agreed on by all stakeholders including NDCP, WHO, non-government organizations, and donors (Appendix 6.10). This summary of recommendations along with the notes from the working groups was then used as one of the resources at future working group meetings to design the Cambodia National Dengue Strategic Plan for 2017-2020. The key recommendations for further research included (1) comparing the cost of activities in the strategic plan with economic costs of dengue, (2) determining how best to integrate routine entomological surveillance (including adult mosquitoes) in high risk locations, (3) determining the best strategies for vector control in urban areas, and (4) evaluating the use of different insecticides and others tools. In addition to the recommendations that came from the project, there were some additional ideas for future research. Some of which have already been started or accomplished.

School-based dengue control programs

A joint proposal between Malaria Consortium and Global Health Asia was submitted to the WHO Special Programme for Research and Training in Tropical Diseases (TDR). The grant opportunity focused on using multidisciplinary teams to develop socio-ecological strategies to

prevent infectious disease. This was based on the trial results, but expanded the focus to include schools and developing a new dengue prevention school curriculum with the Ministry of Education. The proposal was focused around using Social Ecological Systems and Resilience theory to develop transdisciplinary dengue vector control strategies.

The project is now in its second year and is investigating whether a set of disease-specific interventions, including integrated vector management-based source reduction tools (e.g. guppy fish targeting immature mosquitos and community developed mosquito traps targeting adult mosquitoes), COMBI-based health education, and socio-ecological systems approach will significantly reduce mosquito immatures and adults in rural primary schools and households in two districts in Cambodia. While this project also adds to our knowledge on the use of guppy fish for vector control in Cambodia, it also provides important new experience developing community developed mosquito traps with recycled materials. It also is helping to establish new links between the Ministry of Health and the Ministry of Education and will provide inter-ministerial school curriculum that can be adapted for use nationwide in the future. The baseline results have not been made publicly available yet, but information on the key findings is meant to be updated frequently on the public website [18]. There was also a video produced on the project which can be accessed here: https://www.youtube.com/watch?v=U5Sl7TiKQSk.

An additional proposal arose out of this work which looked at the effect of student-controlled interventions, consisting of both mosquito control and education, to reduce dengue and entomological risk factors in the school environment and communities in Yangon, Myanmar. The proposal was selected by the Research Council of Norway's program on Global Health and

Vaccination Research. The project will benefit from the previous experiences in both the trial presented in this PhD and the WHO TDR project mentioned above. This trial is the first cluster randomized trial to examine the effect of using larvivorous fish on dengue incidence and will provide some evidence on how mosquito indices are associated with dengue incidence. The trial protocol has been developed and submitted to the ethics review board in Myanmar for approval. The project plans to have activities running from 2019-2022. It is hoped that the information gained in this project will help fill some knowledge gaps, but also open up new research questions to explore around community and school-based dengue control programs.

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Appendix

Appendix 2.1: PRISMA checklist

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	37
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	37-40
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	41
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	42
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	37
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	42
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	42
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	42-43
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	43-44
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	44
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	44
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	44
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	N/A
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	N/A

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	N/A
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	44
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	44
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	73-76
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figures on page 73-76
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	51-54
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	53
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	53-54
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	54-55

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Appendix 3.1: Informed consent form

ផ្នែកទី ១៖ សន្លឹកព័ត៌មាន

ចំណងជើងការសិក្សា៖ ការអនុវត្តគម្រោងបញ្ចូលគ្នា ក្នុងការគ្រប់គ្រងភ្នាក់ងារចម្លងនៃជំងឺគ្រុនឈាមនៅក្នុងខេត្តកំពង់ចាម នៃប្រទេសកម្ពុជា ។

វត្ថុបំណង៖ គម្រោងនេះផ្តោតលើទិដ្ឋភាពជាច្រើននៃការវាយតំលៃផ្សេងៗគ្នា និង បង្កើតគោលនយោបាយដើម្បីផ្តល់អនុសាសន៍ក្នុងការគ្រប់គ្រងជំងឺគ្រុនឈាមក្នុងប្រទេសកម្ពុជា។

នីតិវិធី៖ យើងនឹងជ្រើសរើសមនុស្សមួយចំនួននៅក្នុងភូមិនេះ ដើម្បីចូលរួមក្នុងការសិក្សាដែលវានឹងជួយឱ្យប្រសើរឡើងនូវយុទ្ធសាស្ត្រថ្នាក់ជាតិសម្រាប់ការគ្រប់គ្រងជំងឺគ្រុនឈាមនៅក្នុងប្រទេសកម្ពុជា ។

យើងខ្ញុំនឹងធ្វើការចាប់មូស ចាប់ដង្កូវទឹក ពីផ្ទះរបស់អ្នក និង សួរសំនួរមួយចំនួនទាក់ទងទៅនឹងការផ្គត់ផ្គង់ទឹកក្នុងផ្ទះរបស់អ្នក ។ ខ្ញុំនឹងផ្តល់ព័ត៌មានជូនអ្នក ហើយខ្ញុំសូមអញ្ជើញអ្នកឲ្យចូលរួមក្នុងការសិក្សានេះ។ មុនពេលអ្នកសម្រេចចិត្តថាតើអ្នកចង់ចូលរួម ឬមិនចង់នោះ អ្នកអាចនិយាយជាមួយនរណាដែលអ្នកមានអារម្មណ៍ថាអ្នកស្រួលនិយាយជាមួយបាន។ អាចមានពាក្យពេចន៍មួយចំនួនដែលអ្នកប្រហែលជាមិនយល់។ សូមប្រាប់ខ្ញុំឲ្យឈប់ក្នុងពេលយើងអានព័ត៌មាននោះ រួចខ្ញុំនឹងពន្យល់ប្រាប់អ្នកនូវចំណុចដែលអ្នកមិនយល់នោះ។ ប្រសិនបើអ្នកមានសំណួរអ្វីនៅពេលក្រោយទៀត អ្នកអាចសួរខ្ញុំ សួរអ្នកឯកទេសសត្វមូស ឬបុគ្គលិកបាន។ អ្នកអាចទាក់ទងទៅកាន់សមាជិកក្រុមស្រាវជ្រាវនៅពេលណាក៏បាន ដោយប្រើប្រាស់ព័ត៌មានសម្រាប់ទំនាក់ទំនងខាងក្រោម៖ **ឈ្មោះ លោក សំ ប៊ុនឡេង ទូរស័ព្ទ៖ 078 696 946 ឬ លោក ឌុំ ឌីណា 092 777 664 ។**

ហានិភ័យ និងផលប៉ះពាល់៖ នឹងមិនមានហានិភ័យណាកើតឡើងទេបន្ទាប់ពីអ្នកបានចូលរួមក្នុងការសិក្សានេះ ។

អត្ថប្រយោជន៍៖ ប្រសិនបើអ្នកចូលរួមក្នុងការសិក្សានេះ អ្នកប្រហែលជាមិនបានទទួលអត្ថប្រយោជន៍ដោយផ្ទាល់ណាមួយឡើយ ប៉ុន្តែការចូលរួមរបស់អ្នកទំនងជានឹងជួយចូលរួមចំណែកដល់ការរុករកវិធីសាស្ត្រនៃការគ្រប់គ្រងជំងឺគ្រុនឈាមនៅក្នុងប្រទេសកម្ពុជា ។

ផ្សេងៗ៖ ពួកខ្ញុំនឹងមិនចែករំលែកពត៌មានរបស់អ្នកចូលរួមក្នុងការសិក្សាជាមួយអ្នកខាងក្រៅឡើយ។ ព័ត៌មានដែលយើងប្រមូលបានពីគម្រោងស្រាវជ្រាវនេះ នឹងត្រូវរក្សាជាការសម្ងាត់តាមកម្រិតដែលច្បាប់អនុញ្ញាត។ ព័ត៌មានអំពីអ្នកដែលយើងនឹងប្រមូលក្នុងអំឡុងពេលការសិក្សានេះនឹងត្រូវរក្សាទុកយ៉ាងល្អ ហើយមិនមាននរណាផ្សេងក្រៅពីអ្នកស្រាវជ្រាវដែលអាចមើលព័ត៌មាននោះបានឡើយ។ ព័ត៌មានទាំងឡាយអំពីរូបអ្នក នឹងមានដាក់លេខនៅលើនោះជំនួសឲ្យឈ្មោះរបស់អ្នក។ មានតែអ្នកស្រាវជ្រាវប៉ុណ្ណោះដែលនឹងដឹងលេខរបស់អ្នក។ ពួកខ្ញុំនឹងមិនចែកព័ត៌មានសម្ងាត់ជាមួយអ្នកខាងក្រៅឡើយ ប៉ុន្តែនៅពេលក្រោយទៅ ពួកខ្ញុំនឹងបោះពុម្ពផ្សាយលទ្ធផលដោយមិនមានបញ្ជាក់អត្តសញ្ញាណរបស់អ្នកឡើយ ដើម្បីឲ្យអ្នកដែលមានចំណាប់អារម្មណ៍អាចរៀនសូត្រពីការស្រាវជ្រាវរបស់យើង និងជួយដល់សហគមន៍ដទៃទៀតបាន។

សំណើនេះ ត្រូវបានពិនិត្យ និងអនុម័តយល់ព្រមដោយគណៈកម្មការសីលធម៌ជាតិ សម្រាប់ការស្រាវជ្រាវក្នុងប្រទេសកម្ពុជា ដែលជាគណៈកម្មការមួយមានទំនួលក្នុងការធានាឲ្យបានថាអ្នកចូលរួមក្នុងការស្រាវជ្រាវបានទទួលការការពារពីគ្រោះថ្នាក់នានា។ ប្រសិនបើអ្នកមានបំណងចង់

ដឹងបន្ថែមទៀតអំពីគណៈកម្មការសីលធម៌ជាតិ សូមទំនាក់ទំនងទៅកាន់ក្រសួងសុខាភិបាល តាមអាស័យដ្ឋានផ្ទះលេខ 151-153 មហាវិថីកម្ពុជាក្រោម ក្រុងភ្នំពេញ។

ការចូលរួមរបស់អ្នក នឹងជួយដល់យើងខ្ញុំ ក្នុងការគ្រប់គ្រងជំងឺគ្រុនឈាម ហើយនឹងជួយដល់សង្គមជាតិ និងមនុស្សជំនាន់ក្រោយទៀតផងដែរ។

ផ្នែកទី ២៖ ការយល់ព្រមដោយផ្អែកលើព័ត៌មានគ្រប់គ្រាន់
ចំណងជើងការសិក្សា៖ ការអនុវត្តគម្រោងបញ្ចូលគ្នា ក្នុងការគ្រប់គ្រងភ្នាក់ងារចម្លងនៃជំងឺគ្រុនឈាម នៅក្នុងខេត្តកំពង់ចាម នៃប្រទេសកម្ពុជា ។

ខ្ញុំត្រូវបានអញ្ជើញឱ្យចូលរួមនៅក្នុងការស្រាវជ្រាវនេះក្នុងគោលបំណងដើម្បីធ្វើការអភិវឌ្ឍវិធីសាស្ត្រដ៏សមស្របសម្រាប់ការគ្រប់គ្រងជំងឺគ្រុនឈាមនៅក្នុងប្រទេសកម្ពុជា ។ ខ្ញុំបានទទួលព័ត៌មានថា ហានីភ័យមានកម្រិតទាប។ ខ្ញុំបានដឹងថា ខ្ញុំនឹងមិនបានទទួលអត្ថប្រយោជន៍ផ្ទាល់ខ្លួនឡើយ។ ខ្ញុំបានទទួលឈ្មោះរបស់អ្នកស្រាវជ្រាវដែលខ្ញុំអាចទាក់ទងបានដោយងាយស្រួលតាមរយៈលេខទូរស័ព្ទ និងអាស័យដ្ឋាន។

ខ្ញុំបានអានព័ត៌មាននេះ ឬមានអ្នកបានអានឲ្យខ្ញុំស្តាប់។ ខ្ញុំមានឱកាសបានសួរសំណួរអំពីព័ត៌មាននេះ ហើយខ្ញុំបានទទួលចម្លើយគួរជាទីពេញចិត្តសម្រាប់សំណួររបស់ខ្ញុំ។ ខ្ញុំយល់ព្រមដោយស្ម័គ្រចិត្ត និងបានដឹងថា ខ្ញុំមានសិទ្ធិដកការយល់ព្រមរបស់ខ្ញុំគ្រប់ពេលវេលា មិនថាដោយហេតុផលអ្វី ដោយមិនមានប៉ះពាល់ដល់ការថែទាំផ្នែកវេជ្ជសាស្ត្ររបស់ខ្ញុំឡើយ។

ឈ្មោះអ្នកចូលរួម៖ _____ ហត្ថលេខាអ្នកចូលរួម៖_____
កាលបរិច្ឆេទ៖_____

ខ្ញុំបានធ្វើជាសាក្សីបញ្ជាក់ថាមានការអានទម្រង់ការយល់ព្រមដោយផ្អែកលើព័ត៌មានត្រឹមត្រូវជូនដល់អនាគតអ្នកចូលរួម ហើយបុគ្គលអ្នកចូលរួមផ្ទាល់ក៏មានឱកាសបានសួរសំណួរផងដែរ។ ខ្ញុំសូមអះអាងថាបុគ្គលនេះបានផ្តល់ការយល់ព្រមដោយសេរី។

ឈ្មោះសាក្សី	_____	ស្នាមមេដៃរបស់អ្នកចូលរួម (ប្រសិនបើអាច)
ហត្ថលេខារបស់សាក្សី	_____	
កាលបរិច្ឆេទ	_____	
ឈ្មោះអ្នកស្រាវជ្រាវ	_____	
ហត្ថលេខាអ្នកស្រាវជ្រាវ	_____	
កាលបរិច្ឆេទ	_____	

Part I: Information Sheet

Title: Implementing Integrated Vector Management for dengue control in Cambodia: community perceptions and policy development

Objectives: This project focuses on several aspects of evaluating alternatives and creating useful policy recommendations for dengue vector control in Cambodia.

Procedures: We are inviting some of the people in this village to take part in this study which will help to improve the national strategy for dengue control.

We are collecting some entomological samples (mosquitoes) from your household and will be asking some questions related to your household water supply. I am going to give you information and invite you to participate in this study. Before you decide whether you want to participate, you can talk to anyone you feel comfortable with. There may be some words that you do not understand. Please ask me to stop as we go through the information, and I will take time to explain. If you have questions later, you can ask me, the study entomologist or the staff. You can contact a member of the investigating team at any time using the contact details below: **Name: Sam Bunleng telephone number: 078 696 946 or Duom Dyna telephone number 092 777 664.**

Your participation in this study is **entirely voluntary**. Even if you agree now, you can decide to change your mind and withdraw yourself later. There will be no negative consequences to you or your family.

Risks and discomforts: There are no any risks will be happen after you take-part in this study.

Benefits: If you participate in this research, there may not be any other direct benefit to you but your participation is likely to help contribute to the search for the best possible dengue control approach in Cambodia.

Other: We will not be sharing the identity of those participating in the research. The information that we collect from this research project will be kept confidential to the extent allowed by law. Information about you that will be collected during the research will be put away and no one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is. Confidential information will not be shared, but later on, we will publish the results which will not identify you in order for other interested people to learn from our research and help other communities.

This proposal has been reviewed and approved by National Ethics Committee for Health Research in Cambodia, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find out more about the National Ethics Committee, contact the Ministry of Health, No 151 – 153 Kampuchea Krom Blvd., Phnom Penh.

Your participation will help us to control dengue and this will benefit society and future generations.

Part II: Informed consent

Title: Implementing Integrated Vector Management for dengue control in Cambodia: community perceptions and policy development

I have been invited to participate in this research that aims to generate the necessary information to develop an appropriate strategy for dengue control in Cambodia.

I have been informed that the risks are minimal. I am aware that there may be no benefit to me personally. I have been provided with the name of a researcher who can be easily contacted using the number and address I was given for that person.

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily and understand that I have the right to withdraw my consent at any time and for any reason without in any way affecting my medical care.

Print Name of Participant _____ Signature of Participant_____ Date _____

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print Name of witness _____

Signature of witness _____
Date _____

Print Name of Researcher _____

Signature of Researcher _____
Date _____

Thumb print of Participant (if applicable)

ផ្នែកទី ១៖ សន្លឹកព័ត៌មាន

ចំណងជើងការសិក្សា៖ ការអនុវត្តគម្រោងបញ្ចូលគ្នា ក្នុងការគ្រប់គ្រងភ្នាក់ងារចម្លងនៃជំងឺគ្រុនឈាម នៅក្នុងខេត្តកំពង់ចាម នៃប្រទេសកម្ពុជា ។

វត្ថុបំណង៖ គោលបំណងនៃការស្រាវជ្រាវរបស់យើង គឺដើម្បីប្រមូលព័ត៌មានដែលចាំបាច់ទាំងឡាយ ដើម្បីបង្កើតឡើងនូវដំណើរការ/យុទ្ធវិធីមួយសម្រាប់ការលុបបំបាត់ជំងឺគ្រុនចាញ់ដ៏សមស្រប ដោយរួមមានការជ្រើសរើសបុគ្គលឆ្លងកាត់ព្រំដែន ដោយប្រើមធ្យោបាយមួយដើម្បីកំណត់នូវសក្តានុពលព្រមទាំងកត្តាហានិភ័យសម្រាប់ជំងឺគ្រុនចាញ់។

នីតិវិធី៖ យើងនឹងជ្រើសរើសមនុស្សមួយចំនួននៅក្នុងភូមិនេះ ដើម្បីចូលរួមក្នុងការសិក្សាដែលវានឹងជួយឱ្យប្រសើរឡើងនូវយុទ្ធសាស្ត្រថ្នាក់ជាតិសម្រាប់ការគ្រប់គ្រងជំងឺគ្រុនឈាមនៅក្នុងប្រទេសកម្ពុជា ។

យើងខ្ញុំនឹងសួរសំណួរមួយចំនួន ដែលទាក់ទងទៅនិងចំណេះដឹង អាកប្បកិរិយា និងការអនុវត្តន៍ការការពារជំងឺគ្រុនឈាម ។ ខ្ញុំនឹងផ្តល់ព័ត៌មានជូនអ្នក ហើយខ្ញុំសូមអញ្ជើញអ្នកឲ្យចូលរួមក្នុងការសិក្សានេះ។ មុនពេលអ្នកសម្រេចចិត្តថាតើអ្នកចង់ចូលរួម ឬមិនចង់នោះ អ្នកអាចនិយាយជាមួយនរណាដែលអ្នកមានអារម្មណ៍ថាអ្នកស្រួលនិយាយជាមួយបាន។ អាចមានពាក្យពេចន៍មួយចំនួនដែលអ្នកប្រហែលជាមិនយល់។ សូមប្រាប់ខ្ញុំឲ្យឈប់ក្នុងពេលយើងអានព័ត៌មាននោះ រួចខ្ញុំនឹងពន្យល់ប្រាប់អ្នកនូវចំណុចដែលអ្នកមិនយល់នោះ។ ប្រសិនបើអ្នកមានសំណួរអ្វីនៅពេលក្រោយទៀត អ្នកអាចសួរខ្ញុំ សួរទៅវេជ្ជបណ្ឌិតរបស់ក្រុមស្រាវជ្រាវ ឬបុគ្គលិកបាន។ អ្នកអាចទាក់ទងទៅកាន់សមាជិកក្រុមស្រាវជ្រាវនៅពេលណាក៏បាន ដោយប្រើប្រាស់ព័ត៌មានសម្រាប់ទំនាក់ទំនងខាងក្រោម៖ **ឈ្មោះ លោកវេជ្ជបណ្ឌិត ប៉ូ លី ទូរស័ព្ទ៖ 011 886 836 ឬ លោកវេជ្ជបណ្ឌិត អ៊ិត សុផល 012 333 771** ។

ហានិភ័យ និងផលប៉ះពាល់៖ នឹងមិនមានហានិភ័យណាកើតឡើងទេបន្ទាប់ពីអ្នកបានចូលរួមក្នុងការសិក្សានេះ ។

អត្ថប្រយោជន៍៖ ប្រសិនបើអ្នកចូលរួមក្នុងការសិក្សានេះ អ្នកប្រហែលជាមិនបានទទួលអត្ថប្រយោជន៍ដោយផ្ទាល់ណាមួយឡើយ ប៉ុន្តែការចូលរួមរបស់អ្នកទំនងជានឹងជួយចូលរួមចំណែកដល់ការរុករកវិធីសាស្ត្រនៃការគ្រប់គ្រងជំងឺគ្រុនឈាមនៅក្នុងប្រទេសកម្ពុជា ។ ការផ្តល់សំណងប៉ះប៉ូវដូចជាត្រីខកំប៉ុង នឹងត្រូវផ្តល់ជូនអ្នកចូលរួម។

ផ្សេងៗ៖ ពួកខ្ញុំនឹងមិនចែករំលែកពត៌មានរបស់អ្នកចូលរួមក្នុងការសិក្សាជាមួយអ្នកខាងក្រៅឡើយ។ ព័ត៌មានដែលយើងប្រមូលបានពីគម្រោងស្រាវជ្រាវនេះ នឹងត្រូវរក្សាជាការសម្ងាត់តាមកម្រិតដែលច្បាប់អនុញ្ញាត។ ព័ត៌មានអំពីអ្នកដែលយើងនឹងប្រមូលក្នុងអំឡុងពេលការសិក្សានេះ នឹងត្រូវរក្សាទុកយ៉ាងល្អ ហើយមិនមាននរណាផ្សេងក្រៅពីអ្នកស្រាវជ្រាវដែលអាចមើលព័ត៌មាននោះបានឡើយ។ ព័ត៌មានទាំងឡាយអំពីរូបអ្នក នឹងមានដាក់លេខនៅលើនោះជំនួសឲ្យឈ្មោះរបស់អ្នក។ មានតែអ្នកស្រាវជ្រាវប៉ុណ្ណោះដែលនឹងដឹងលេខរបស់អ្នក។ ពួកខ្ញុំនឹងមិនចែកព័ត៌មានសម្ងាត់ជាមួយអ្នកខាងក្រៅឡើយ ប៉ុន្តែនៅពេលក្រោយទៅ ពួកខ្ញុំនឹងបោះពុម្ពផ្សាយលទ្ធផលដោយមិនមានបញ្ជាក់អត្តសញ្ញាណរបស់អ្នកឡើយ ដើម្បីឲ្យអ្នកដែលមានចំណាប់អារម្មណ៍អាចរៀនសូត្រពីការស្រាវជ្រាវរបស់យើង និងជួយដល់សហគមន៍ដទៃទៀតបាន។

សំណើនេះ ត្រូវបានពិនិត្យ និងអនុម័តយល់ព្រមដោយគណៈកម្មការសីលធម៌ជាតិ សម្រាប់ការស្រាវជ្រាវក្នុងប្រទេសកម្ពុជា ដែលជាគណៈកម្មការមួយមានទំនួលក្នុងការធានាឲ្យបានថាអ្នកចូលរួមក្នុងការស្រាវជ្រាវបានទទួលការការពារពីគ្រោះថ្នាក់នានា។ ប្រសិនបើអ្នកមានបំណងចង់ដឹងបន្ថែមទៀតអំពីគណៈកម្មការសីលធម៌ជាតិ សូមទំនាក់ទំនងទៅកាន់ក្រសួងសុខាភិបាល តាមអាស័យដ្ឋានផ្ទះលេខ 151-153 មហាវិថីកម្ពុជាក្រោម ក្រុងភ្នំពេញ។

ការចូលរួមរបស់អ្នក នឹងជួយដល់យើងខ្ញុំ ក្នុងការប្រយុទ្ធប្រឆាំងជំងឺគ្រុនចាញ់ ហើយនឹងជួយដល់សង្គមជាតិ និងមនុស្សជំនាន់ក្រោយទៀតផងដែរ។

ផ្នែកទី ២៖ ការយល់ព្រមដោយផ្អែកលើព័ត៌មានគ្រប់គ្រាន់
ចំណងជើងការសិក្សា៖ ការអនុវត្តគម្រោងបញ្ចូលគ្នា ក្នុងការគ្រប់គ្រងភ្នាក់ងារចម្លងនៃជំងឺគ្រុនឈាមនៅក្នុងខេត្តកំពង់ចាម នៃប្រទេសកម្ពុជា ។

ខ្ញុំត្រូវបានអញ្ជើញឱ្យចូលរួមនៅក្នុងការស្រាវជ្រាវនេះក្នុងគោលបំណងដើម្បីធ្វើការអភិវឌ្ឍវិធីសាស្ត្រដ៏សមស្របសម្រាប់ការគ្រប់គ្រងជំងឺគ្រុនឈាមនៅក្នុងប្រទេសកម្ពុជា ។ ខ្ញុំបានទទួលព័ត៌មានថា ហានីភ័យមានកម្រិតទាប។ ខ្ញុំបានដឹងថា ខ្ញុំនឹងមិនបានទទួលអត្ថប្រយោជន៍ផ្ទាល់ខ្លួនឡើយ។ ខ្ញុំបានទទួលឈ្មោះរបស់អ្នកស្រាវជ្រាវដែលខ្ញុំអាចទាក់ទងបានដោយងាយស្រួលតាមរយៈលេខទូរស័ព្ទ និងអាស័យដ្ឋាន។

ខ្ញុំបានអានព័ត៌មាននេះ ឬមានអ្នកបានអានឲ្យខ្ញុំស្តាប់។ ខ្ញុំមានឱកាសបានសួរសំណួរអំពីព័ត៌មាននេះ ហើយខ្ញុំបានទទួលចម្លើយគួរជាទីពេញចិត្តសម្រាប់សំណួររបស់ខ្ញុំ។ ខ្ញុំយល់ព្រមដោយស្ម័គ្រចិត្ត និងបានដឹងថា ខ្ញុំមានសិទ្ធិដកការយល់ព្រមរបស់ខ្ញុំគ្រប់ពេលវេលា មិនថាដោយហេតុផលអ្វី ដោយមិនមានប៉ះពាល់ដល់ការថែទាំផ្នែកវេជ្ជសាស្ត្ររបស់ខ្ញុំឡើយ។

ឈ្មោះអ្នកចូលរួម៖ _____ ហត្ថលេខាអ្នកចូលរួម៖_____
កាលបរិច្ឆេទ៖_____

ខ្ញុំបានធ្វើជាសាក្សីបញ្ជាក់ថាមានការអានទម្រង់ការយល់ព្រមដោយផ្អែកលើព័ត៌មានត្រឹមត្រូវជូនដល់អនាគតអ្នកចូលរួម ហើយបុគ្គលអ្នកចូលរួមផ្ទាល់ក៏មានឱកាសបានសួរសំណួរផងដែរ។ ខ្ញុំសូមអះអាងថាបុគ្គលនេះបានផ្តល់ការយល់ព្រមដោយសេរី។

ឈ្មោះសាក្សី	_____	ស្នាមមេដៃរបស់អ្នកចូលរួម (ប្រសិនបើអាច)
ហត្ថលេខារបស់សាក្សី	_____	
កាលបរិច្ឆេទ	_____	
ឈ្មោះអ្នកស្រាវជ្រាវ	_____	
ហត្ថលេខាអ្នកស្រាវជ្រាវ	_____	
កាលបរិច្ឆេទ	_____	

Appendix 3.2: SPIRIT 2013 checklist: recommended items to address in a clinical trial protocol and related documents

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Title page, p. 80
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	ISRCTN, p.82
	2b	All items from the World Health Organization Trial Registration Data Set	see Registry ISRCTN. P.82
Protocol version	3	Date and version identifier	Title page, p. 80
Funding	4	Sources and types of financial, material, and other support	Acknowledgement p. 109
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Title page, p. 80/ Authors' contributions, p.109/Acknowledgement, p.109

	5b	Name and contact information for the trial sponsor	Malaria Consortium - Development House, 56-64 Leonard Street, London, United Kingdom EC2A 4LT, p.109
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Acknowledgement, p. 109-110
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Data Monitoring section, pg. 102
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Introduction p. 83-88
	6b	Explanation for choice of comparators	Introduction p. 83-88
Objectives	7	Specific objectives or hypotheses	Introduction, p. 89
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	p.97

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Study setting, p. 90
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Eligibility criteria, p. 90
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Interventions, p. 90-91
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Data Monitoring, p. 102
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	adherence, p. 94
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Outcomes, p. 95
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Interventions pg. 121 and figure 3.3

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Sample size, p. 96
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Adherence, p. 94
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Allocation, p. 97
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Allocation, p.97
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Allocation, p. 97
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Data collection methods, p. 97-100
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Adherence, Pg. 94
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Data management, p. 102
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	p. 102
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Methods for any additional analyses, p. 102
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Handling of missing data, p. 102
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Data Monitoring, p. 102

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Monitoring, p.102
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Monitoring, p. 102
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Monitoring, p. 102
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Research ethics approval, p. 107
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	Research ethics approval, p. 107
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Research ethics approval, p. 107
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Research ethics approval, p. 107
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Competing interests, p. 107

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Access to data, p. 103
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Ancillary and post-trial care Pg. 103
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Dissemination policy, p. 103
	31b	Authorship eligibility guidelines and any intended use of professional writers	Dissemination policy, p. 103
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Access to data, p. 103
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 3.1
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Biological specimens, pg. 108

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons “Attribution-NonCommercial-NoDerivs 3.0 Unported” license.

Appendix 4.1: CONSORT checklist

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	126
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	127-128
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	130-133
	2b	Specific objectives or hypotheses	133
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	91-92
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	None
Participants	4a	Eligibility criteria for participants	134
	4b	Settings and locations where the data were collected	134
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	91-95
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	135-138
	6b	Any changes to trial outcomes after the trial commenced, with reasons	None
Sample size	7a	How sample size was determined	137-138
	7b	When applicable, explanation of any interim analyses and stopping guidelines	None
Randomisation:			138
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	138

Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	138
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	138
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and h	138
	11b	If relevant, description of the similarity of interventions	140-142
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	140-141
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	140-141
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	167
	13b	For each group, losses and exclusions after randomisation, together with reasons	167
Recruitment	14a	Dates defining the periods of recruitment and follow-up	138
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	162
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	167
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	161-164
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	161-164
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	none
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	145
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	149-150
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	150
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	146-151
Other information			
Registration	23	Registration number and name of trial registry	128

Protocol	24	Where the full trial protocol can be accessed, if available	136
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	152

Appendix 4.2: Community health worker monthly monitoring form

CHW Monthly Monitoring								
CHW Name		Date						
Village		Number of Health Education Sessions						
List Any Adevere Events Reported								
House ID No	Large Water Jars (>50L)				Small Water Jars (<50L)			
	Total Jars	Number < 2 Guppies	Number of Guppies Added	How many days each week do you use large jars?	Total Jars	Number with PPF	Number of PPF Added	How many days each week do you use small jars?
1								
2								
3								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								

Appendix 4.3: Entomology survey forms

Indoor Resting Adult Summary Form

					Date		DD		MM		YY				
Village	name		code		Collector 1 name										
					Collector 2 name										
					Supervisor name										
	Fill in field					**Fill in laboratory**									
House ID	No, people sleep in HH last night	Name of HH	Cup IDs	Adult mosquitos		No. of adult mosquitoes									Comments
				Present	Absent	Aegypti		Albo		Culex	Anopheles	Other	Total		
						M	F	M	F						

Container Characterisation Form

					Date	DD		MM		YY		
Village	name		code		Collector 1 name							
Household	name		code		Collector 2 name							
					Supervisor name							
Fill in new forms for >4 containers per household [use a new form(s) for more than 4 containers]												
Container category		Container IDs	Total container	**Container no. 1**		**Container no. 2**		**Container no. 3**		**Container no. 4**		
				diameter or (LxW)	height (cm)	diameter or (LxW)	height (cm)	diameter or (LxW)	height (cm)	diameter or (LxW)	height (cm)	
Drum (D)	Fish											
	None											
Water storage jar (J)	Fish											
	PPF											
	None											
Concrete tank (CT)	Fish											
	None											
Cement basin (CB)	Fish											
	None											
Small pot, <10 L (P)	Fish											
	PPF											
	None											
Flower vase/tray (F)	Fish											
	PPF											
	None											
Tyre (T)	Fish											
	PPF											
	None											
Can/bottle (C)	Fish											
	None											
Miscellanious - Domestic Use (M)	Fish											
	PPF											
	None											
Others (O)	Fish											
	PPF											
	None											

Housing Variables

Village	name		code		Date	DD		MM		YY	
Household	name		code		Collector 1 name						
GPS Coordinate	N:				Collector 2 name						
	E:				Supervisor name						

Q1. House Elevation	On Stilts	1
	Ground level	2
Q2. House Type	Cement	1
	Wood	2
	Cement/Wood	3
	Other	4
Q3. Exterior of Home Painted	No	0
	Yes	1
Q4. Interior of Home Painted	No	0
	Yes	1
Q5. Window curtains	No	0
	Yes	1
Q6. Ownership of Bednets	None	0
	Conventional Net	1
	ITN	2
	LLIN	3

Q6. Uses Bednets	No	0
	Yes	1
Q7. Toilet Type	Flush	1
	Latrine	2
	Other	98
Q8. Water Supply	Piped	1
	Well or Bore	2
	Rain Harvest	3
	Other	98
Q9. Main Construction Material	Wood	1
	Conrregated Iron	2
	Concrete/stone	3
	Other	98

Premise Condition Index

Village	name		code		Date	
Household	name		code		Collector 1 name	
					Collector 2 name	
					Supervisor name	

Premise variables	Description	Classification score
P1. House condition	Well maintained, eg newly painted or new house	**1**
	Moderately well-maintained house	**2**
	Not well-maintained house, eg paint peeling, broken items visible, dilapidated old house	**3**
P2. Yard condition	Tidy yard, eg no rubbish or trash evident, well-maintained gardens and lawn	**1**
	Moderately tidy yard	**2**
	Untidy yard, rubbish and trash abundant and the garden or lawn with overgrown grass	**3**
P3. Shade condition	Very little or no shade (<25%), eg no major trees or bush	**1**
	Some shade (>25 but <50%)	**2**
	Plenty of shade, >50%, eg large trees evident, layers of shrubs, green house, plastic tarp sheet or overhanging roofs used	**3**
P4. Water supply and storage	Piped water supply only	**1**
	Well water supply only	**2**
	Rain water and/or river water	**3**

Appendix 4.4: KAP Survey

កម្រងសំនួរសម្រាប់ខ្នងផ្ទះ

Questionnaire_HH_Final

អត្តសញ្ញាណកម្ម IDENTIFICATION
លេខកូដសិក្សាខ្នងផ្ទះ **|____|____|____|____|**
Household Study ID Number

Q1. លេខទូរស័ព្ទ (ខ្នងផ្ទះ) Tel: (+855)_____	**Q4. GPS (location in GPS name)** _____ X: _____ Y: _____
Q2. ខេត្ត |___| Province name/code: _____	**Q5. ការិយាល័យសុខាភិបាលស្រុកប្រតិបត្តិ** |___|___| OD name/code:_____
Q3. ភូមិ |__|__|__|__| Village name/code:	**Q6. ឈ្មោះអ្នកសំភាសន៍** |___|___| Name of Interviewer/code:

សេចក្តីណែនាំ: ជម្រាបសួរ ខ្ញុំបាទ នាងខ្ញុំឈ្មោះ_____ខ្ញុំធ្វើការជាមួយក្រសួងសុខាភិបាល និងអង្គការម៉ាឡារាខុនសតធៀម ដើម្បីមករៀនសូត្រ និងស្វែងយល់ស្តីពីវិធីនៃការការពារជំងឺគ្រុនឈាម។ យើងខ្ញុំចង់រៀនពីការយល់ដឹង ឥរិយាបទ និង វិធីការពារនានាដែលអ្នកបានអនុវត្តន៍នៅផ្ទះ។ ដើម្បីសិក្សារៀនសូត្រពីវិធីនេះ យើងខ្ញុំសូមសួរនូវសំណួរ មួយចំនួនដូចខាងក្រោម។ យើងខ្ញុំនឹងធ្វើការអង្កេតពីទីកន្លែងបង្កកំណើតរបស់មូស និងវិធីសាស្ត្រនៃការការពារនៅក្នុង និងក្រៅផ្ទះរបស់អ្នក។ យើងខ្ញុំនឹងសួរចំនួន២០នាទី។ ការចូលរួមឆ្លើយសំនួររបស់អ្នកគឺរក្សាដោយសំងាត់ និងដោយ ស្ម័គ្រចិត្ត។ បើអ្នកយល់ព្រមឆ្លើយសំនួរ អ្នកក៏អាចបដិសេធ ឬក៏ឈប់ឆ្លើយសំនួរក៏បាន។ រាល់ចំលើយរបស់អ្នកនឹងជួយដល់យើងខ្ញុំក្នុងការអភិវឌ្ឍន៍នូវការបង្ការ និងទំនួលខុសត្រូវចំពោះជំងឺគ្រុនឈាមក្នុងប្រទេសកម្ពុជា។ ជាងនេះទៅទៀត នឹងជួយដល់ការបង្កើតនូវចំណេះដឹង ដែលអ្នកនឹងរៀនពីវិធីដ៏មានតម្លៃដើម្បីការពារជំងឺគ្រុនឈាម និងកត្តាជំងឺចម្លង នានានៅក្នុងផ្ទះ និងសហគមន៍របស់អ្នក។ សូមគិតថានេះមិនមែនជាការស្វែងរកចំឡើយខុសត្រូវឡើយ។ តើអ្នកមានសំនួរអ្វីទេ? បើគ្មានសំនួរ សូមអនុញ្ញាតិឲ្យសួរ?

ហត្ថលេខា/ឈ្មោះ (Signature/Name)**:** _____ ថ្ងៃទីខែឆ្នាំ (Date)**:** _____ (DD/MM/YYYY)

អ្នកបញ្ចូលទិន្នន័យ (Data entry) 1st : _____ ថ្ងៃទីខែឆ្នាំ (Date): _____ (DD/MM/YYYY)

2nd: _____ ថ្ងៃទីខែឆ្នាំ (Date): _____ (DD/MM/YYYY)

ផ្នែកទី ១ ស្ថានភាពសេដ្ឋកិច្ចប្រជាសាស្ត្រ Section 1: Socio-demographics

ល/រ. No.	សំណួរ QUESTION	ប្រភេទលេខកូដ CODING CATEGORIES		រំលង Skip
Q101	**តើអ្នកមានអាយុប៉ុន្មាន?** How old are you?	**ចំនួនឆ្នាំ**: |____|____| (Age)		
Q102	**ភេទអ្វី?** What is your gender?	**ប្រុស** (Male)	1	
		ស្រី (Female)	2	
Q103	**តើអ្នកមានដើមកំណើតជាជនជាតិអ្វី?** What ethnic group do you identify with? **(ចម្លើយមានតែមួយ)** **(Only 1 answers)**	**ខ្មែរ** (Khmer)	1	
		ចាម (Cham)	2	
		វៀតណាម (Vietnamese)	3	
		ផ្សេងៗ បញ្ជាក់ _____ Other (specify)	98	
Q104	**តើអ្នកទទួលបានការអប់រំខ្ពស់បំផុតកម្រិតណា?** What was the highest level of school attended by you/household head completed? **(ចម្លើយមានតែមួយ)** **(Only 1 answers)**	**មិនដែលបានរៀន** (None)	0	
		សាលាក្រៅផ្លូវការ (រៀននៅវត្ត.....) (Unofficial school)	1	
		បឋមសិក្សា (1-6) (Primary school, 1-6)	2	
		អនុវិទ្យាល័យ (7-9) (Secondary school, 7-9)	3	
		វិទ្យាល័យ (10-12) (High school, 10-12)	4	
		ថ្នាក់មហាវិទ្យាល័យ (University level)	5	
Q105	**តើមុខរបរអ្វីដែលជាចំណូលចម្បងសម្រាប់ទំនុក បម្រុងក្នុងគ្រួសារ?** What is your main occupation? **(ចម្លើយមានតែមួយ)** **(Only 1 answers)**	**គ្មានមុខរបរ ឬ នៅផ្ទះ** None/Stay at home	0	
		កសិករ Farmer	1	
		បុគ្គលិកមន្ត្រីរាជការស៊ីវិល Civil government staff	2	
		បុគ្គលិកមន្ត្រីរាជការ ប្រដាប់អាវុធ (ប៉ូលីស/ទាហាន...) Security government staff	3	
		បុគ្គលិកក្រុមហ៊ុនឯកជន Company staff	4	

ល/រ. No.	សំណួរ QUESTION	ប្រភេទលេខកូដ CODING CATEGORIES			រំលង Skip
		បុគ្គលិកមន្ត្រីក្រៅរដ្ឋាភិបាល NGO Staff		5	
		កម្មករលក់កម្លាំងពលកម្ម Labor worker		6	
		អ្នកលក់ដូរតាមផ្ទះ ឬផ្សារ Sell vender or market seller		7	
		ផ្សេងៗ_____ Other		98	
Q106	**តើក្នុងផ្ទះរបស់អ្នកមានសម្ភារៈប្រើប្រាស់ទាំងអស់នេះដែរឬទេ?** Does your household have **ចម្លើយអាចមានច្រើន** **Multiple Answers**		**Yes**	**No**	
		អគ្គិសនី (បណ្តាញអគ្គិសនី រដ្ឋ ឬឯកជន) Electricity (electricity cable state or private)	1	0	
		វិទ្យុ Radio	1	0	
		ទូរទស្សន៍ TV	1	0	
		ទូរស័ព្ទ Mobile Phone	1	0	
		ទូរទឹកកក Refrigerator	1	0	
		ទូញ្ជ ខោអាវ A Wardrobe	1	0	
		ម៉ាស៊ីនដេរ A sewing machine or loom	1	0	
		ក្បាលចាក់ឌីស A CD/DVD/MP3 player	1	0	

ល/រ. No.	សំណួរ QUESTION	ប្រភេទលេខកូដ CODING CATEGORIES			រំលង Skip
		ម៉ាស៊ីនភ្លើង អាគុយ ឬ បន្ទះប្រើពន្លឺព្រះអាទិត្យ Generator/ Battery/Solar power	1	0	
		ផ្សេងៗ _____ Other	1	0	

Q107	**តើមានសម្ភារៈអ្វីខ្លះ នៅក្នុងផ្ទះរបស់អ្នក ដូចខាងក្រោម?** Does any member of this household own: **ចម្លើយអាចមានច្រើន** **Multiple Answers**		**Yes**	**No**	
		នាឡិការដៃ Hand Watch	1	0	
		កង់ Bicycle/cyclo	1	0	
		ម៉ូតូ Motorcycle/scooter	1	0	
		ម៉ូតូកង់បី Motorcycle-cart	1	0	
		គោ ក្របី Oxcart/Horse cart	1	0	
		ឡាន Car/Truck/Van	1	0	
		ទូកមានម៉ាស៊ីន Boat with motor	1	0	
		ទូក Boat without motor	1	0	
		ផ្សេងៗ _____ Other	1	0	
Q108	**តើអ្នកប្រើចំហេះអ្វីខ្លះ សម្រាប់ចំអិនម្ហូបអាហារ នៅក្នុងផ្ទះរបស់អ្នក** What type of fuel does your household use for cooking? **ចម្លើយអាចមានច្រើន** **Multiple Answers**		Yes	No	
		អគ្គីសនី Electricity	1	0	
		ចង្ក្រានហ្គាស LPG	1	0	
		ឡឌស្ទ័ន/ជីវៈឧស្ម័ន Biogas	1	0	
		ធ្យូង Charcoal	1	0	
		ឧស្ស Wood	1	0	

		ចម្បើង ឬ ស្លឹករុក្ខជាតិ Straw/shrubs/grass	1	0	
		គ្រាប់ធញ្ញជាតិ (ឧ. ស្រូលពោត....) Agriculture crop	1	0	
		លាមក សត្វ Animal Dung	1	0	
		គ្មានអាហារចំអិនក្នុងផ្ទះ No food cooked in household	1	0	
		ផ្សេងៗ_____	1	0	

ផ្នែកទី ២ ចំណេះដឹងស្តីពីជំងឺក្រុនឈាម Section 2: Knowledge about dengue

ល/រ. No.	សំណួរ QUESTION	ប្រភេទលេខកូដ CODING CATEGORIES			រំលង Refuse
Q201	តើជំងឺក្រុនឈាម ឆ្លងដោយសារអ្វី? How is dengue transmitted?	មូសខាំ *Aedes* Mosquito bite		1	
		ផ្សេងទៀត បញ្ជាក់_____ Other (Specify)		98	
		មិនដឹង Don't know		99	→Q204
Q202	តើភាគច្រើនមូសបង្កជំងឺក្រុនឈាម (មូសខ្លា) ខាំនៅពេលណា? When do dengue mosquitos **most often** bite? (ចម្លើយមានតែមួយ) **(Only 1 answers)**	មូសខាំ នៅពេលថ្ងៃ Bite during the day		1	
		មូសខាំ នៅពេលយប់ Bite during the night time		2	
		មិនដឹង Don't know		99	
Q203	តើមូសអាចបង្កើតកូននៅកន្លែងណាខ្លះ? Where can mosquitos breed? ចម្លើយអាចមានច្រើន **Multiple Answers**		**Yes**	**No**	
		មិនដឹង Don't know	1	0	
		ក្នុងទឹកពាង Water storage jars	1	0	
		ក្នុងទឹក ដក់ក្នុងសំបកដូង កំប៉ុង Coconut shells /Cans	1	0	
		ក្នុងអាងដូតទឹក (Cement baths)	1	0	
		ទឹកដក់ក្នុងសំបុកត្រោស ឬ ប្រហោងតូចៗលើដើមឈើជុំវិញផ្ទះ Ant traps	1	0	
		ទឹកដក់ក្នុងកន្លែងដែលអាចដក់ទឹកបាននៅជុំវិញផ្ទះរបស់អ្នក Anything with water around your environment	1	0	
		ក្នុងសំបកកង់ឡាន ម៉ូតូ (Tires)	1	0	
		ផ្សេងទៀត បញ្ជាក់ _____	1	0	
Q204	តើអ្នកធ្វើដូចម្តេចខ្លះ ដើម្បីការពារមូសមិនអោយបង្កើតកូនតទៅទៀតបាន? How can you prevent mosquitos from breeding?		**Yes**	**No**	
		មិនដឹង Don't know	1	0	
		ដាក់ថ្នាំអាបែត Use Abate	1	0	

ល/រ. No.	សំណួរ QUESTION	ប្រភេទលេខកូដ CODING CATEGORIES			រំលង Refuse
	អាចមានចំលើយច្រើន *ចូរគូសរង្វង់ជុំវិញចំលើយទាំងអស់* *ចូរសួរដេញរកតើមានអ្វីផ្សេងទៀតឬអត់?* MULTIPLE RESPONSES POSSIBLE CIRCLE ALL MENTIONED PROBE ONCE: ANYTHING ELSE?	**ប្រើ PPF** Use PPF	1	0	
		ប្តូរទឹកក្នុងពាងញឹកញាប់ Changing stored water frequently	1	0	
		ផ្កាប់ធុងចាស់ៗចុះក្រោម Turn containers upside down	1	0	
		ប្រើគំរបពាង Put lids on water jars	1	0	
		ដាក់ត្រីក្នុងពាង Put fish in water jars	1	0	
		ជាញ់ស្ព្រាយ (ថ្នាំមូស) Spraying insecticide	1	0	
		ផ្សេងទៀត_____ Other (Specify)	1	0	
Q205	**ដើម្បីកុំអោយមូសខាំ តើអ្នកការពារខ្លួន ឬ កំសមាជិកអ្នកគ្រួសាររបស់អ្នក ដោយរបៀបណា?** How can you prevent mosquitos from biting you or your family? *អាចមានចំលើយច្រើន* *ចូរគូសរង្វង់ជុំវិញចំលើយទាំងអស់* *ចូរសួរដេញរកតើមានអ្វីផ្សេងទៀតឬអត់?* MULTIPLE RESPONSES POSSIBLE CIRCLE ALL MENTIONED PROBE ONCE: ANYTHING ELSE?		Yes	No	
		មិនដឹង Don't know	1	0	
		ដុត ឬ កប់ សំបកដូង Burn/Bury Coconut Shells	1	0	
		ស្លៀកពាក់ខោអាវវែងៗ Wear long sleeves/long pants	1	0	
		លាបថ្នាំកំចាត់មូស Use mosquito repellent	1	0	
		ដេកក្នុងមុងពេលដេកថ្ងៃ Use mosquito net during day	1	0	
		កាប់ស្មៅគុម្ពោតស៊ប់ទ្រុបនៅជិតផ្ទះ Cut down bushes near the house	1	0	

ល/រ. No.	សំណួរ QUESTION	ប្រភេទលេខកូដ CODING CATEGORIES			រំលង Refuse
		ឲ្យក្មេងៗលេងឆ្ងាយពីជម្រកមូស Have children play far from mosquito breeding areas	1	0	
		ដុតធូបមូសពេលថ្ងៃ Use mosquito coils during the day	1	0	
		សំអាតជុំវិញបរិវេណផ្ទះ Keep household environment clean	1	0	
		បត់ខោអាវឲ្យមានរបៀប Keep cloths tidy	1	0	
		ប្រដាប់ឆក់មូស Electricity trap	1	0	
		ប្រើកង្ហារដេញមូស Use fan	1	0	
		ផ្សេង_____	1	0	
Q206	**តើរោគសញ្ញាជំងឺគ្រុនឈាមមានអ្វីខ្លះ?** What are the symptoms of dengue? ***អាចមានចំលើយច្រើន*** ***ចូរគូសរង្វង់ជុំវិញចំលើយទាំងអស់*** ***ចូរសួរដេញឮកតើមានអ្វីផ្សេងទៀតឬអត់?*** **MULTIPLE RESPONSES POSSIBLE** **CIRCLE ALL MENTIONED** **PROBE ONCE: ANYTHING ELSE?**		Yes	No	
		មិនដឹង (Don't know)	1	0	
		គ្រុនក្តៅ (Fever)	1	0	
		ឈឺក្បាល (Headache)	1	0	
		សន្លឹម (Somnolence)	1	0	
		ក្អួត ចង្អោរ (Nausea/vomiting)	1	0	
		កន្ទួលក្រហមៗ (Rash)	1	0	
		ឈឺស្អឹងឆ្អែង និងចុកចាប់ Aches and Pains/Body pain	1	0	
		ធ្លាក់ឈាម (Bleeding)	1	0	
		អាការៈស្ទើរសន្លប់ Shock	1	0	
		ឈឺសាច់ដុំ Muscular Pain	1	0	
		ផ្សេងៗ _____ Other (Specify)	1	0	

ល/រ. No.	សំណួរ QUESTION	ប្រភេទលេខកូដ CODING CATEGORIES		រំលង Refuse
Q207	**តើអ្នកដឹងពី ពេលដែលជម្ងឺ ក្រុនឈាមកើតឡើង ដែរ ឬ ទេ នៅក្នុងពេលមួយឆ្នាំៗ?** Are there certain times a year when you recognize that more people in your family /village get sick of dengue?	**ទេ** No	0	**→Q301**
		ជាទ Yes	1	
		មិនដឹង Don't know	99	
Q208	**តើជម្ងឺក្រុនឈាមកើតឡើងនៅពេលណា? (ខែណា ដល់ខែណា)** If yes, when?	**ចាប់ពីខែ: _____ ដល់ខែ: _____** **From: ..... to .....**		
		មិនដឹង Don't know	99	

ផ្នែកទី៣៖ឥរិយាបថក្នុងការស្វែងរកសេវាសុខភាព (**Section 3: Health Seeking Behaviour**)

ល/រ. **No.**	សំណួរ **QUESTION:**	ប្រភេទលេខកូដ **CODING CATEGORIES**		រំលង **Refuse**
Q301	**បើមានសមាជិកគ្រួសាររបស់អ្នកក្រុនក្តៅ តើអ្នកនឹងធ្វើអ្វីមុនគេបង្អស់?** If you think you or someone in your family has fever, what would you do **First?** **(ចម្លើយមានតែមួយគត់)** **(Only 1 answer)**	**សេវាសុខភាពសាធារណៈ(រដ្ឋ)** Go to Health Facility	1	
		ពេទ្យឯកជន (Go to Private Provider)	2	
		អ្នកស្ម័គ្រចិត្តសុខភាពភូមិ Go to Community Health Worker	3	
		ទិញថ្នាំនៅហ្វាម៉ាស៊ី (Take Drugs From Pharmacy)	4	
		សំរាកនៅផ្ទះ ឬ រងចាំរហូតជាត់ ក្រុនក្តៅដោយខ្លួនឯង Stay at home/wait for fever to go away	5	
		ផ្សេងៗ _____	6	
		មិនដឹង (Don't know)	99	
Q302	**ប្រសិនបើមានសមាជិកគ្រួសារណាម្នាក់មានរោគ សញ្ញាក្រុនក្តៅ តើរយៈប៉ុន្មានថ្ងៃទើបអ្នកទៅរកការព្យាបាល?** If your family member gets fever, how many days do you wait to seek care after symptoms start?	|___|___| **កត្រាលេខ 0 ប្រើសិនបើក្នុងថ្ងៃក្រុនក្តៅ** **កត្រាលេខ ៩៩ ប្រសិនបើមិនដឹង** Record **"0"** if they respond in the same day Record **"99"** if they respond Don't Know		
Q303	**បើសិនជាអ្នកសង្ស័យថានណាម្នាក់កើតជម្ងឺក្រុនឈាម តើកន្លែងណាដែលអ្នកនឹងណែនាំឬក៏ទៅធ្វើរោគវិនិច្ឆ័យ**? If you suspect someone in your family has dengue, where would you go for advice/testing? **(ចម្លើយមានច្រើន)** **(Multiple answers)**		**Yes**	**No**
		សេវាសុខភាពសាធារណៈ(រដ្ឋ) Go to Health Facility	1	0
		ពេទ្យឯកជន Go to Private Provider	1	0
		ទៅរកអ្នកស្ម័គ្រចិត្តសុខភាពភូមិ Go to Community Health Worker	1	0
		ទិញថ្នាំនៅហាងលក់ថ្នាំ (Pharmacy)	1	0
		ទៅជួបអ្នកដឹកនាំសាសនា (ព្រះសង្ឃ អាចារ្យ ឬ អ្នកផ្តល់ ស្តោះជាដើម) Religious Leader	1	0
		ផ្សេង _____	1	0

ផ្នែកទី ៤ ឥរិយាបថ (**Section 4: Attitude)**

ល/រ. **No.**	សំណួរ **QUESTION:**	ប្រភេទលេខកូដ **CODING CATEGORIES**		រំលង **Refuse**
Q401	**តើជំងឺក្រុនឈាម ជាជំងឺធ្ងន់ធ្ងរមែនដែរ ឬ ទេ?** Dengue is a serious illness?	**ទេ** No	0	

ល/រ. **No.**	សំណួរ **QUESTION:**	ប្រភេទលេខកូដ **CODING CATEGORIES**		រំលង **Refuse**
		ចាទ Yes	1	
		មិនដឹង Don't know	99	
Q402	**តើអ្នកពិតជាប្រឈមនឹងគ្រោះថ្នាក់ដែរ ឬ ទេ នៅពេលកើតជំងឺគ្រុនឈាម?** You are at risk of getting dengue	**ទេ** No	0	
		ចាទ Yes	1	
		មិនដឹង Don't know	99	
Q403	**តើជំងឺគ្រុនឈាមអាចបង្ការមុនបានដែរឬ ទេ?** Dengue fever can be prevented	**ទេ (**No)	0	
		ចាទ (Yes)	1	
		មិនដឹង (Don't know)	99	

ផ្នែកទី ៥ ការអនុវត្តន៍ (Section 5: Practices)

ល/រ. No.	សំណួរ QUESTION:	ប្រភេទលេខកូដ CODING CATEGORIES			វាលង Skip
Q501	តើអ្នកប្តូរទឹក (ពាង អាង....) ញឹកញាប់ប៉ុណ្ណា? How often do you change the storage water? (ចម្លើយមានតែមួយ) (Only 1 answers)	មិនដែរប្តូរសោះ Not at all	0		
		ច្រើនជាងមួយអាទិត្យម្តង More than once a week	1		
		ម្តងក្នុងមួយអាទិត្យ Once time per week	2		
		ពីរដងក្នុងមួយខែ Twice per month	3		
		ច្រើនជាង ១ ខែម្តង More than 1 time per time	4		
		ផ្សេងៗ _____ Other (Specify)	5		
Q502	តើអ្នកមានពាងទឹកធំៗ ចាប់ពី ៥០ លីត្រ ចំនួនប៉ុន្មាន? ហើយមានគម្រប ប៉ុន្មាន? ចំនួនពាងដាក់ត្រី? How many large water jars (>50L) do you have and how many are covered	1.ចំនួនពាង |_____|_____| Number of Jars			
		2.ចំនួនគម្របពាង |_____|_____| Number covered			
		3. ចំនួនពាងដាក់ត្រី |_____|_____| សូមរំលងទៅសំនួរ Q504 ប្រសិនបើចម្លើយ ស្មើសូន្យ "00" Number of jars put fish If record "00" go to Q504			
Q503	បើសិនជាត្រីងាប់ តើអ្នកទៅយកនៅទីណា? If the fish die where would go to replace them? (ចម្លើយមានច្រើន) (Multiple answers)		Yes	No	
		មិនដាក់ថែម Not Replace Them	1	0	
		នៅមណ្ឌលសុខភាព Health Center	1	0	
		នៅផ្ទះអ្នកស្ម័គ្រចិត្តសុខភាពភូមិ Community Health Worker	1	0	
		ផ្សេង ..... Other (specify)	1	0	
		មិនដឹង Don't Know	1	0	
Q504	តើអ្នកស្គាល់ PPF ដែរ ឬ ទេ? Do you know what PPF is?	ទេ No	0		
		ចាទ/ចា Yes	1		
Q505	តើអ្នកធ្វើដូចម្តេចខ្លះជាមួយសំបកដូង ឬ វត្ថុដែល អាច ដក់ទឹកបាន? What do you do with empty coconuts or containers? (ចម្លើយមានតែមួយ) (Only 1 answers)	អត់ធ្វើអ្វីទាំងអស់ Nothing	0		
		ផ្តាប់ចោល Turn them upside down	1		
		ដុត ឬ ច្រកទុក Burn them/put them in trash bag	2		
		ផ្សេងៗ _____ Other (Specify)	3		
Q506	តើអ្នកចងមុងឬទេ នៅពេលសម្រាកពេលថ្ងៃ? How often do you use mosquito nets during daytime naps?	ចំនួនថ្ងៃចងមុង ក្នុង 1 សប្តាហ៍ |____| day/week			
Q507	តើជាទម្លាប់អ្នកស្លៀកខោអាវវែងៗនៅពេលថ្ងៃញឹកញាប់ កម្រិតណាក្នុង ១ សប្តាហ៍ ? How often do you wear long sleeves/long	ចំនួនថ្ងៃស្លៀកពាក់ខោអាវវែង ក្នុង 1 សប្តាហ៍ |____| day/week			

ល/រ. No.	សំណួរ QUESTION:	ប្រភេទលេខកូដ CODING CATEGORIES		រំលង Skip
	pants during the day time?			
Q508	**តើអ្នកប្រើធូកមូសពេលថ្ងៃញឹកញាប់កម្រិតណា?** How often do you use mosquito coils during the day time?	**ចំនួនថ្ងៃប្រើធូកមូសពេលថ្ងៃ ក្នុង 1 សប្តាហ៍** |____| day/week		
Q509	**តើអ្នកគិតថា បរិស្ថានក្នុងសហគមន៍ និងជុំវិញផ្ទះរបស់អ្នកស្អាត ដែរ ឬ ទេ?** Do you think your community and household's environment keep clean?	**ទេ** No	0	
		បាទ/ចា Yes	1	
		មិនដឹង Don't Know	99	
Q510	**តើអ្នកគិតថា សម្លៀកបំពាក់ក្នុងផ្ទះរបស់អ្នករៀបចំបានត្រឹមត្រូវដែរ ឬទេ ? (សង្កេត)** Do you think the cloths in your household keep tidy?	**ទេ** No	0	
		បាទ/ចា Yes	1	
		មិនដឹង Don't Know	99	

បញ្ចប់បទសំភាសន៍ END INTERVIEW
សូមថ្លែងអំណរគុណដល់អ្នកដែលបានចំណាយពេលចូលរួមក្នុងការសំភាសន៍នេះ។

Thank respondent for taking the time to be interviewed.

Appendix 6.1: Determining the specificity and sensitivity of Dengue Duo Rapid Diagnostic Tests

Methods

Search strategy and eligibility criteria

This review was carried out between January and February 2015. All studies reporting on the sensitivity and specificity of the Dengue Duo test were eligible for inclusion.

Data sources and search strategy

Studies were identified by searching PubMed, scanning reference lists of articles and consultation with experts in the field. No limits were applied for language in case there was an available English translation. The only search term used was "Dengue duo".

Study selection

Titles and abstracts were imported into Endnote (Thompson Reuters, Philadelphia, PA, USA), duplicates were removed, and the remaining records were screened. Full texts of potentially relevant records were retrieved and assessed for eligibility, contacting the author of the report as necessary. Reference lists of all potentially eligible articles and reviews were also searched.

Results

Search results

Initially 34 records were identified through database searches. After screening of the title and abstracts, the remaining 20 papers were assessed and reviewed in full, after which 10 articles were excluded. A total of 10 studies were then included in the review (Table 1).

The included studies were published between 2012 and 2015. Nine of the studies had original data and one was a meta-analysis. The sensitivity ranged from 58-96% and the specificity ranged

from 83-99%. However, four studies did not report what the used as the gold standard for comparison, and the ones who did mention it did not use a standardized method. Therefore, there are limitations to comparing the results across studies. Regardless, the results show that it may be difficult use RDTs in place more accurate lab-based diagnostics.

Table 1: Results from search on specificity and sensitivity of SD Dengue Duo

Author	Type	Year	Country	Senstivity	Specificity	Gold standard
Andries		**2012**	Cambodia	85.7-94.4	**83.9-90**	laboratory diagnosis was based on RT-PCR, isolation of DENV after inoculation into mosquito cell lines, detection of anti-DENV IgM and measure of an increase of anti-DENV antibodies titer measured by hemagglutination inhibition assay (HIA) between acute and convalescent sera.
	IgM			72.7	89.4	Dengue virus infections were confirmed on an individual patient basis, with the paired admission and convalescent-phase specimens tested by the AFRIMS with IgM and IgG
	NS1			48.5	99.4	
Blacksell	IgM and NS1	**2011**	Sri Lanka	92.9	88.8	
Carter		**2015**	Cambodia	58	85	The Panbio Japanese Encephalitis Dengue IgM Combo ELISA was retrospectively used for reference serology
Gan		**2014**	Singapore	93	92	Plasma samples were subject to a two-stage real-time reverse transcriptase polymerase chain reaction comprising screening using SYBR green followed by a tetraplex probe-based serotype detection assay [18]. The serological suite used was: Platelia NS1 ELISA (Bio-Rad Laboratories, Marnes-la-Coquette, France), PanbioH Dengue IgG Indirect, IgG Capture, and IgM Capture ELISAs (Alere Inc., Waltham, MA, USA)
	NS1		Asia and	59	NA	laboratory confirmed by the presence of DENV detected by RT-PCR and/or virus isolation
Hunsperger	IgM	**2014**	Americas	89-98	NA	
MMWR		**2013**	Micronesia	66	NA	RT-PCR and anti-DENV IgM capture ELISA
Osorio		**2010**	Columbia	80.7	89.1	Viral culture, nested RT-PCR or paired IgM
Pal		**2015**	Venezuela, Cambodia, and United States	87.3	86.8	NA
Pal		**2014**	Central America	72.4	95	Virus isolation was attempted for all acute samples, and DENV was identified using serotype-specific IFAs. DENV IgM and IgG titers were determined by ELISA.
Sanchez-Vargas		**2013**	Mexico	90.65	89.66	All serum samples were tested and diagnosed with 3 reference ELISA techniques: Platelia Dengue NS1 Ag Test (Bio-Rad Laboratories, Marnes-la-Coquette, France), overall sensitivity 91% and specificity 100%; Panbio IgM Capture ELISA (Panbio Diagnostics, Brisbane, Australia), overall sensitivity 94.7% and specificity 100%; and IgG capture ELISA (Panbio Diagnostics), overall sensitivity 96.3% and specificity 91.4%. These ELISA kits were used following the guidelines of InDRE.
	NS1			62.4	100	
	NS1/IgM			75.5	100	
Tricou	NS1/IgM /IgG	**2010**	Vietnam	83.7	97.9	
	NS1			58	NA	NA
Valdez Sandoval	IgM	**2012**	Cuba	96	98.4	NA
Zhang		**2014**	Meta Analysis	71	99	NA

Appendix 6.2: Determining the use of Dengue Rapid Diagnostic Tests in Sero Surveys

Methods

Search strategy and eligibility criteria

This review was carried out between January and February 2015. All studies reporting on the use of Rapid Diagnostic Tests for dengue sero surveys were eligible for inclusion.

Data sources and search strategy

Studies were identified by searching PubMed, scanning reference lists of articles and consultation with experts in the field. No limits were applied for language in case there was an available English translation. The search terms used are found in Table 1.

Table 1: Search terms used in the review

dengue seroconversion
dengue sero epidemiology
dengue sero
dengue sero*

Study selection

Titles and abstracts were imported into Endnote (Thompson Reuters, Philadelphia, PA, USA), duplicates were removed, and the remaining records were screened. Full texts of potentially relevant records were retrieved and assessed for eligibility, contacting the author of the report as necessary. Reference lists of all potentially eligible articles and reviews were also searched.

Results

Search results

Initially 624 records were identified through database searches. After screening of the title and abstracts, the remaining 48 papers were assessed and reviewed in full, after which 35 articles were excluded. A total of 13 studies were then included in the review (Table 1).

The included studies were published between 2011 and 2015. None of the studies used RDTs in dengue sero surveys.

Table 1: Results from search on tests used in dengue sero surveys

Authors	Year	Test used
Anders	2015	RT-PCR, NS1-ELISA
Andayi	2014	A cross-sectional ELISA and sero-neutralisation-based sero-epidemiological analysis
Domingo	2011	A short nucleotide fragment located in the carboxyl terminus of the dengue E gene was used for the characterization of DENV strains and the identification of their sero- and genotype
Fox	2014	Dengue virus-reactive immunoglobulin G enzyme-linked immunosorbent assay
Leder	2013	Indirect enzyme-linked immunosorbent assay (ELISA), Dengue IgG Indirect ELISA and Dengue IgM Capture ELISA (PanBio Diagnostics, Brisbane, QLD, Australia). This assay has reported sensitivities in non-endemic populations as follows: IgM in primary infection of 94.7% (95% CI: 85.4–98.91%); IgM in secondary infection of 55.7% (95% CI 46.6–64.7%); IgG in primary infection of 91.4% (95% CIs not provided); IgG in secondary infection of 97% (range: 73.8–99.7%) (http://panbiodengue.com/product/dengue-igg-indirect-elisa; http://www.alere.com/us/en/product-details/panbio-dengue-igg-indirect-elisa.html). The assay's specificity is reported to be close to 100% (range: 91–100%) (http://panbiodengue.com/product/dengue-igg-indirect-elisa; http://www.alere.com/us/en/product-details/panbio-dengue-igg-indirect-elisa.html)
Liebman	2012	Infection status was determined by seroconversion based on plaque neutralization testing of sequential blood samples taken at approximately six-month intervals, with date of infection assigned as the middate between paired samples
Martins	2014	Indirect ELISA technique
Mazaba-Liwewe	2014	Differential antibody tests were done by ELISA
Poudel	2012	Enzyme linked immunosorbent assay (ELISA) kit
Pun	2011	Enzyme linked immunosorbent assay
Soghaier	2014	The samples were analyzed using Panbio ELISA kits (DF IgG indirect)
Tiexeira	2012	The techniques used consisted of ELISA for the detection of IgG antibodies and modified haemagglutination inhibition (HI) assay
Visser	2013	For DFV Pan Bio® kits (Inverness Medical Innovations Australia Pty Ltd, Queensland, Australia) were used (''Dengue IgM Capture ELISA'': 95% sensitivity and 100% specificity and ''Dengue IgG Indirect ELISA'': 98% sensitivity and 100% specificity

Appendix 6.3: Ecologists raise alarm over releases of mosquito-killing guppies

For decades, health officials have used guppies (*Poecilia reticulata*) for mosquito control. H. KRISP

Ecologists raise alarm over releases of mosquito-killing guppies

By Kelly Servick | Oct. 25, 2016 , 7:15 PM

The little guppy *Poecilia reticulata* has developed a big reputation. For decades, the fish has been championed as a mosquito fighter and dumped into ponds and ditches to eat up the insect's larvae. But among scientists, it has a different reputation—as an invasive species with a remarkable ability to reproduce and spread.

“It all sounds like it’s magical—you put the guppies in, they eat the mosquitoes, everything is fine,” says Rana El-Sabaawi, an ecologist at the University of Victoria in Canada and lead author on the new paper. “Our concern is that you have a potentially invasive species that is being introduced haphazardly.”

Larva-gobbling guppies may have been cutting-edge technology for U.K. colonialists aiming to rid the empire of mosquitoes at the turn of the century. But to El-Sabaawi, the strategy seems so old-fashioned that she was surprised to find out large-scale projects are underway. While “randomly Googling guppies,” she came across news reports from Pakistan that health officials had released thousands of the fish into the ponds and sewers of Karachi in 2013 to fight the transmission of dengue fever. And in a **widely circulated news video** documenting Zika control efforts in Brazil, El-Sabaawi was troubled by footage of a municipal government worker apparently “wandering around with a bunch of guppies and basically just introducing them in ditches.”

That’s unnerving for El-Sabaawi and her co-authors because they know guppies are efficient invaders. They’re hearty and fertile, surviving in relatively polluted water, reproducing often, and giving birth to fast-growing, live young. A combination of accidental aquarium releases and mosquito control projects have spread the species from its native range in the Caribbean and the northern coast of South America to at least 69 countries, according to a **2011 survey**.

And several studies suggest that introduced guppies threaten biodiversity. Researchers in Hawaii found that guppies released in the 1920s drove down native fish populations, perhaps by competing with them for food and living space, and had likely changed the cycle of nutrients in water: Guppy-rich areas showed increased levels of dissolved nitrogen—from ammonium in fish urine and gill excretions—which, in turn, stimulated algae growth. (Another fish commonly used in mosquito control—*Gambusia affinis*—has also been associated with declines in native fish species.)

John Hustedt, senior technical officer of the nonprofit Malaria Consortium in Phnom Penh, which has been releasing the fish into water storage jars in rural households to combat dengue fever and other mosquito-transmitted diseases. Hustedt hopes that a study his group has just completed will provide new evidence for the guppies' value. Preliminary results showed that reductions in the number of adult mosquitoes were two times greater in households with guppies than in those without.

"If someone comes out and says, 'Actually it doesn't work and it's going to cause you a problem,' that can decrease the chance that the government would be more open to trying [guppy release] on a large scale," he says.

As for ecological risks, guppies in isolated containers may be less likely to spread than those dumped into urban sewers and ditches. But Hustedt also questions the distinction between native and nonnative for a species that is already so ubiquitous. The guppies used in his project were found in a farm in a province outside Phnom Penh; their original source is unknown. "It seems to me that they've been here for quite a long time, and they're already in the environment," he says.

Although the benefits and risks of guppy releases may be highly context-dependent, some researchers are simply taking a hard line. "The use of fish to control mosquito disease vectors should be abandoned by authorities," says Valter Azevedo-Santos, an ichthyologist at São Paulo State University in Botucatu, Brazil, who co-authored **a letter objecting to the strategy** published in *Science* earlier this year. He believes resources would be better spent on other control measures: insecticides, sanitary measures such as eliminating standing water in homes, and even the **experimental release of genetically engineered mosquitoes** to spread a lethal gene. As health workers cast around for ways to combat Zika, he hopes this paper will give them pause. "This mismanagement must be abandoned, or new fish invasions will occur in the near future," he says. "This is a special moment."

Posted in: **Biology**, **Plants & Animals**
doi:10.1126/science.aal0304

Kelly Servick
Kelly is a staff writer at *Science*.
Email Kelly | Twitter

More from News

Appendix 6.4: PROSPERO registration

PROSPERO International prospective register of systematic reviews

Review title and timescale

1 Review title
Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.
Impact on the ecosystem associated with introduction of guppy fish(*Poecilia reticulata*) : a scoping review

2 Original language title
For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.
(not applicable)

3 Anticipated or actual start date
Give the date when the systematic review commenced, or is expected to commence.
31 July, 2018

4 Anticipated completion date
Give the date by which the review is expected to be completed.
31 December, 2018

5 Stage of review at time of this submission
Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started ×

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	No	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

Review team details

6 Named contact
The named contact acts as the guarantor for the accuracy of the information presented in the register record.
John Hustedt

7 Named contact email
Enter the electronic mail address of the named contact.
johnhustedt@gmail.com

8 Named contact address
Enter the full postal address for the named contact.
London School of Hygiene & Tropical Medicine
Keppel Street
London
WC1E 7HT

9 Named contact phone number
Enter the telephone number for the named contact, including international dialing code.
+1 (404) 969-5609

10 Organisational affiliation of the review
Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.
London School of Hygiene and Tropical Medicine
Website address:

http://www.lshtm.ac.uk/

11 Review team members and their organisational affiliations
Give the title, first name and last name of all members of the team working directly on the review. Give the organisational affiliations of each member of the review team.

Title	First name	Last name	Affiliation
Mr	John	Hustedt	LSHTM
Dr	Leigh	Bowman	Umea University
Dr	Leo	Barak	Malaria Consortium
Dr	Pierre	Echaubard	Global Health Asia
Dr	John	Bradley	LSHTM
Dr	Jeffrey	Hii	ABT associates
Dr	Olaf	Horstick	Universität Heidelberg
Dr	Neal	Alexander	LSHTM

12 Funding sources/sponsors
Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.
Malaria Consortium provided some funding to the first author for this review.

13 Conflicts of interest
List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.
Are there any actual or potential conflicts of interest?
None known

14 Collaborators
Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

Title	First name	Last name	Organisation details

Review methods

15 Review question(s)
State the question(s) to be addressed / review objectives. Please complete a separate box for each question.
Determine any potential impact on the ecosystem associated with the introduction of guppy fish(*Poecilia reticulate)*

16 Searches
Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.
Studies will be identified by searching electronic databases, scanning reference lists of articles and consultation with experts in the field. No limits will be applied for language in case there was an available English translation of the full text. If no translation was available only English and Spanish articles will be evaluated. The search will be applied to Pubmed, EMBASE, Web of Science, LILACS, Global Health, and the Cochrane Database of Systematic Reviews. Potential search terms include:
Ecosystem AND gupp*
Environment AND gupp*
Nitrogen AND gupp*
Algae AND gupp*
Density AND gupp*
Impact AND gupp*
Ecosystem AND *Poecilia reticulata*
Environment AND *Poecilia reticulata*
Nitrogen AND *Poecilia reticulata*
Algae AND *Poecilia reticulata*
Density AND *Poecilia reticulata*

Impact AND *Poecilia reticulata*

17 URL to search strategy
If you have one, give the link to your search strategy here. Alternatively you can e-mail this to PROSPERO and we will store and link to it.

I give permission for this file to be made publicly available
Yes

18 Condition or domain being studied
Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.
Dengue is the most rapidly spreading mosquito-borne viral disease in the world, with a 30 fold increase in incidence over the past 50 years and an expansion into new geographic areas. Dengue infection is caused by bites of infected *Aedes* mosquitoes, principally *Aedes aegypti*. Dengue has a wide clinical spectrum that ranges from asymptomatic infection to death. With an estimated 3.6 billion people in 124 countries at risk of contracting the disease and 390 million dengue infections occurring each year (of which 96 million are clinically apparent) the dengue virus has become a leading cause of illness and death in the tropics and subtropics. Due to the rise in dengue cases, and the current lack of effective vaccines and therapeutics there is an urgent need to identify more effective vector control methods. Recent studies have assessed the efficacy of using larvivorous fish, especially guppy fish, for dengue vector control. The benefits of using guppies includes cultural acceptance in certain regions, low costs of implementation, and potential for community based actions independent from national level intervention. However, recent articles have highlighted the invasion of guppies into local ecosystems and potential impacts on biodiversity and the ecosystem more broadly following their use.

Use of guppy fish for mosquito control has been implemented around the world for more than a hundred years. In response to the recent Zika outbreaks in 2015-16, several countries recommended using larvivorous fish for control of *Aedes* mosquitoes. Many biologists and ecologists were concerned about the impact this may have on the ecosystem and expressed concern guppies may invade non-native ecosystems, deplete native fauna, and alter ecosystems. Guppies are known to be highly plastic and acclimate to new environments while reproducing frequently. In one recorded case, a single pregnant female was able to establish a guppy population. This can result in invasion of guppies into previously naive environments. This extremely resilient nature is what has attracted many scientists to study guppies and use them to establish biological models within conservation biology. However, some studies have shown effects on nitrogen levels in water, reduction in algae levels, or effects on resident fish densities, However, the extent of these impacts on the ecosystem does not seem to be uniform or been evaluated in an independent review. As the potential to invade local ecosystems has already been well established in the affirmative, the review will focus on the effects of invasion rather than the potential of invasion itself.

19 Participants/population
Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.
All populations will be considered for inclusion.

20 Intervention(s), exposure(s)
Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed
All studies reporting the impact on the ecosystem through introduction of guppies will be eligible for inclusion.

21 Comparator(s)/control
Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).
There are no alternatives being compared in this review.

22 Types of study to be included
Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.
Only studies reporting primary data (including qualitative information) will be included.

23 Context
Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

24 Primary outcome(s)
Give the most important outcomes.
As this is a scoping review, it will address the exploratory research question aimed at mapping key concepts, types of evidence, and gaps in research related to a defined area or field by systematically searching, selecting, and synthesizing existing knowledge. Therefore, the primary outcome may be further defined after searching, but initially the primary outcome is expected to focus on the potential impact on biodiversity and the ecosystem associated with introduction of guppies (e.g. algae and nitrogen levels, and changes in native organisms).

Give information on timing and effect measures, as appropriate.

25 Secondary outcomes
List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.

Give information on timing and effect measures, as appropriate.

26 Data extraction (selection and coding)
Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.
We will develop a data extraction sheet, pilot test it on randomly selected included studies, and refine it accordingly. One review author will extract data and the second author will check the extracted data. Disagreements will be resolved by discussion between the two review authors; if no agreement could be reached, it is planned a third author will decide.

27 Risk of bias (quality) assessment
State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.
As this is a scoping review no quality assessment will take place.

28 Strategy for data synthesis
Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.
As this is a scoping review no data synthesis will take place.

29 Analysis of subgroups or subsets
Give any planned exploration of subgroups or subsets within the review. 'None planned' is a valid response if no subgroup analyses are planned.
None Planned

Review general information

30 Type and method of review
Select the type of review and the review method from the drop down list.
Intervention, Systematic review
Infections and infestations, International development, Public health (including social determinants of health)

31 Language

Select the language(s) in which the review is being written and will be made available, from the drop down list. Use the control key to select more than one language.

English

Will a summary/abstract be made available in English?

Yes

32 Country

Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Use the control key to select more than one country.

Cambodia, Colombia, Germany, United Kingdom, Sweden

33 Other registration details

Give the name of any organisation where the systematic review title or protocol is registered together with any unique identification number assigned. If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here.

N/A

34 Reference and/or URL for published protocol

Give the citation for the published protocol, if there is one.

N/A

Give the link to the published protocol, if there is one. This may be to an external site or to a protocol deposited with CRD in pdf format.

I give permission for this file to be made publicly available

Yes

35 Dissemination plans

Give brief details of plans for communicating essential messages from the review to the appropriate audiences.

A paper will be submitted to a leading journal in this field. Furthermore, should the findings of the review warrant a change in practice, a summary report will be prepared and sent to relevant stakeholders.

Do you intend to publish the review on completion?

Yes

36 Keywords

Give words or phrases that best describe the review. (One word per box, create a new box for each term)

Vector Control
Guppy
Larvivorous Fish
Ecology
Dengue
Systematic Review

37 Details of any existing review of the same topic by the same authors

Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

N/A

38 Current review status

Review status should be updated when the review is completed and when it is published.

Ongoing

39 Any additional information

Provide any further information the review team consider relevant to the registration of the review.

This review is being undertaken as part of the planning potential policy changes related to a cluster randomized trial to compare different dengue vector options (including guppies) in Cambodia.

Appendix 6.5: WHO Supports targeting *Aedes* mosquito larvae through integrated vector management in Cambodia

World Health Organization | Western Pacific

WHO Supports targeting Aedes mosquito larvae through Integrated Vector Management in Cambodia

11 October 2017

WHO provided technical assistance and supporting Malaria Consortium to piloting a project on integrated vector management to assess the effectiveness of various control strategies to prevent the transmission of dengue. The study was conducted in Kampong Cham province, Cambodia and was funded by the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH commissioned by the Federal Ministry for Economic Cooperation and Development (BMZ) and UK aid from the UK government.

Kampong Cham is one of the high-risk provinces, recording several dengue outbreaks in recent years. Cases can skyrocket, especially during the rainy season, where the environment provides mosquitos with more breeding sites and human movements play a major role in the spread of the disease.

Sample larvae and pupae were collected during the entomological survey.
© Malaria Consortium

Overcrowding also contributes to increased transmission in the area, and so a larger population is at risk of the *Aedes* mosquitoes bites and diseases transmitted by them. In 2016, the Western Pacific Regional Office (WPRO) provided technical support to a local project piloted by the Malaria Consortium, which aimed to reduce dengue transmission in this densely-populated province.

Although there is evidence suggesting that the use of guppy fish can be beneficial in dengue vector control, WPRO and the Malaria Consortium conducted a survey to evaluate the efficiency of the initiative. WPRO also supported a community assessment on local dengue practices, which included training for community health workers on behavior change communication and health education. The larval control exercise was also combined with behaviour change communication and health education to the community under integrated vector management.

Inspection of the number and condition of guppy fish in water jars.
©Malaria Consurtium

Appendix 6.6: Summary of Recommendations

Dengue Integrated Vector Management Project Dissemination and Policy Uptake Workshop and Key Stakeholders Meeting
Dec, 1-2, Phnom Penh, Cambodia

Attendees at Key Stakeholders Meeting:
CNM: Bunleng Sam
WHO: Rabindra Romauld Abeyasinghe, Tang Choon Siang, Prof. Juan Arredondo-Jimenez, Vibol Chan, Luciano Tuseo
MC: John Hustedt, Yves Bourny, Sergio Lopes
IPC: Sebastien Boyer
GIZ: Maylin Meincke
GEF: Shannon Conlon

1. **Policy, Strategy and Funding**

Revise and update plan of action and policy documents over next 3 months (Deadline for end of March):

- **National Dengue Strategic Plan** (Situation Analysis, Objectives, Strategic Implementation, Action Plan and Indicator Monitoring Framework, Coordination Mechanisms, Detailed Budget)
    - Strategic plan will be harmonized with WHO Regional Strategic Plan
    - Mapping funding gaps identified in detailed budget to implement the National Strategic Plan (possibly look at climate funding)
    - Strengthen community based vector control activities
    - Communications strategy for private sector (Hotels / Private sector vector control companies) - Business analysis to make case for alternative methods
    - WHO will provide technical assistance and GIZ can support the elaboration process of the documents
- **Epidemic Preparedness and Response Plan**
    - Possibility GEF will fund modeling to predict dengue cases (early warning system) in late 2017; actual funding may only be available in 2018.
- **Dengue Guideline for Diagnosis and Treatment**
    - WHO consultant (Professor Lucy Lam) will come to assist in finalizing D&T guideline
    - A training document needs to be developed
    - 1 -2 Training of Trainers workshops should be conducted
    - WHO recommendation to test at least 10% of cases at each level by RDT - Possibility of GEF funding for expansion of RDTs for diagnosis and treatment

- **Dengue Surveillance guidelines** (based on strategic plan)
    - Need to integrate available data
    - Issue with funding for sentinel sites (CNM lab costs could be much cheaper than using IPC exclusively)
    - Possibility of GEF funding to expand high quality clinical diagnosis for adults in additional sentinel sites
    - Integrating data from CNM/IPC/CDC/NAMRU-2 should be considered
    - Possibility of adding data from Mobile Malaria Workers or other CHWs should be considered
- **Cambodia Business Plan for Dengue Control** (Dengue Economic Impact in Cambodia and Costing)
    - Comparing cost of strategic plan to economic costs of dengue resulting in Return on Investment (ROI) for Cambodia
    - Consider raising funds for health economist to finish business plan by March
    - GIZ could possibly support if done by April/May
    - TOR/budget needs to be developed by 15 Jan and sent to GIZ

2. Operational Challenges for Vector Control

- Routine and outbreak response needed at lower levels to understand and respond correctly
    - Surveillance system strengthening needed
- Strengthening proactive approach (including routine vector control in parallel with outbreak response)
- Increase capacity of human resources in advance of outbreaks, use WHO recommended tools, and ensure they are accepted and used in the field (including participation by actors at CNM/PHD/OD/village level)
- Routine entomological surveillance (including adult mosquitoes) needs to conducted in high risk locations
- Community owned vector control needs to emphasized
- Vector control in rural/urban areas needs to targeted separately

3. Available Tools for vector control

- Need for integrated strategy – encourage moving away from dependence on one product or method
- Abate was found to be more expensive than other insecticides in cost effectiveness models and was found to be resistant in various areas around the country tested (Banteay Meanchey, Battambong, Kampong Cham, Kampot, Kandal, Kratie, Phnom Penh, and Tbong Khmum)

- Deltamethrin/Permethrin was found to be resistant in all areas tested (Phnom Penh, Kampong Cham, Battambong, Siem Reap) – mapping of vector susceptibility needed in all provinces suggested to identify where it might still not be resistant
- Need to evaluate different insecticides/other tools
- Insecticides should only be used for outbreak control not for routine prevention
- Routine prevention should include source reduction (e.g. guppy fish)
- Community based interventions should be encouraged

Appendix 6.7: Dengue in Cambodia: using guppies and growth hormones to fight disease

OCTOBER 09, 2017 - BY ALISON BOOTH

DENGUE IN CAMBODIA: USING GUPPIES AND GROWTH HORMONES TO FIGHT DISEASE

Image courtesy of the Malaria Consortium

Dengue in Cambodia is endemic all year round. But budgets are tight. For some time, The World Health Organization (WHO) and Asian Development Bank (ADB) have been helping the authorities search for a cost-effective and sustainable way to tackle dengue in Cambodia. A more recent research project (funded by UKAID and Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) with WHO as a partner) combined guppy fish, a larvicide based on disrupting growth hormones and COMBI (Communication for Behavioral Impact) activities.

Traditionally, Cambodian's fight against dengue involves using chemicals to control the Aedes aegypti mosquito. But fogging hasn't always had the desired impact. In 2015, for instance, there were 15,000 cases of dengue in Cambodia.

Over the years, researchers have tried other ways of reducing the Aedes population, with limited success:

- Initially releasing Mesocyclops (a crustacean that preys on mosquito larvae) into water containers where Aedes larvae live looked promising, but larvae numbers crept up over time. Also, people didn't like crustaceans.
- Bacillus thuringiensis israelensis (Bti), a soil-dwelling bacterium, were found to significantly reduce larvae numbers in containers with river and well water, but only for two or three months.
- Jar covers with long-lasting insecticidal netting (LN) treated with deltamethrin eliminated two-thirds of adult Aedes, but they degraded over time, and children regularly used them as toys.

HUNGRY FOR LARVAE

One other approach, however, has had more success at decreasing the number of Aedes mosquitoes in Cambodia: guppy fish.

Between April 2006 and April 2007, a USAID-funded project tested the use of guppy fish in domestic water containers in 14 villages in the Kampong Speu province of Cambodia. Local volunteers bred and distributed the fish. On average, each guppy ate more than 100 mosquito larvae each day, significantly reducing the number of Aedes mosquitoes. After a year, only 10% of containers with guppies contained mosquitoes, compared with 50% of containers without fish.

Image via Malaria Consortium

"Guppies live quite well in Cambodia's very hot and dry conditions," says John Hustedt epidemiologist for the Malaria Consortium in Cambodia. "And they eat whatever larvae are in the water container."

Following this success, the WHO and ADB funded a larger-scale IVM Guppy Fish Project, combining guppy fish with Communication for Behavioral Impact (COMBI) activities. COMBI uses communication and mobilization to improve the community's behavior – rather than just attitude – towards water use and vector-borne disease prevention.

"They wanted to learn how to mobilize the community," said John. "And to help the community understand how and why they're using guppies."

TACKLING SMALLER BREEDING SITES

The project, however, had its limitations: other Aedes breeding sites, including containers too small for guppies to survive in, still needed to be tackled.

A product based on Pyriproxyfen (PPF), a growth hormone that prevents juvenile Aedes mosquitoes from developing into adults, looked promising. When studied in Cambodia in 2003, it stopped 90% of Aedes larvae maturing for 20 weeks. During the study, researchers placed a controlled-

release formulation of pyriproxyfen consisting of cylindrical resin strands in concrete water storage jars between 400 and 500 liters in size.

However, maker Sumitomo Chemical never released this specific solution for technical reasons. The company has since reformulated the PPF-based solution, developing and releasing the slow-release formulation called SumiLarv® 2 MR.

The local community can use this controlled-release disk in water jars too small for guppy fish while cutting the cost of larviciding since it only needs to be distributed once every six months – the whole rainy season. John explains: "Normally, you'd put larvacide pellets into water containers up to six times a year. This controlled-release disk can, therefore, save on operational costs."

Added to that, SumiLarv® 2 MR can work at very low levels. "It doesn't kill the mosquitoes; it's just a growth inhibitor that messes with the mosquitoes' hormones," says John.

A COMBINED APPROACH

John and his team initiated a trial to study how effective combining guppies, PPF and COMBI activities would be. The study site included approximately 6,000 households, divided into three groups:

1. Guppies, PPF and COMBI activities
2. Guppies and COMBI activities
3. Standard vector control activities from the Ministry of Health

Groups one and two placed two guppy fish into water containers bigger than 50 liters; their COMBI activities included health education sessions, posters, banners, t-shirts, and songs. Group one also placed one SumiLarv® 2 MR disk in containers of between 10 and 50 liters.

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Image via Malaria Consortium

GUPPIES ACCEPTED WELL

Local people tended to like using the guppies, even if they hadn't used them before. Not only are fish were seen as lucky, but the people of Cambodia traditionally use fish for health interventions.

Even though previous studies have shown guppies don't significantly increase e.coli or other bacteria in water jars, a few people – mainly foreigners – still questioned the practice. "We talked to them about how there were fish in the lake where they get their water," says John. "It was then no longer an issue."

John is also very conscious that some outsiders are concerned that the guppies could upset the balance of the local ecosystem. "While introducing a non-native fish species into the ecosystem could hurt the local fish and deplete oxygen levels, guppies have been here in Cambodia for many years," says John. "I haven't seen any peer-reviewed scientific evidence of harm in Cambodia."

"I think it's a valid concern, which we should think about; but without evidence to the contrary, I don't think it's a reason why we shouldn't use

guppies," John continues. "You could potentially distribute only fish from one sex, so they don't breed if they do get into the water."

RELIGIOUS CONCERNS

Local people, however, were more concerned about using the PPF devices. "We created COMBI messages that local volunteers could use to explain that PPF is at a very low level that doesn't kill the larvae and isn't harmful to people," says John. "After a while, their reservations were gone."

People's main concern, however, was grounded in their religion. Older people with strong religious beliefs didn't want to use guppies or PPF to kill the larvae because they were living creatures and it would be frowned upon. "We explained that, while it's important for us to treat living creatures nicely, if you don't get rid of the mosquitoes then dengue transmission will continue and some children will die – even children in your community," says John. "We told them, 'You have to choose between getting rid of the larvae or people becoming sick and possibly dying.'"

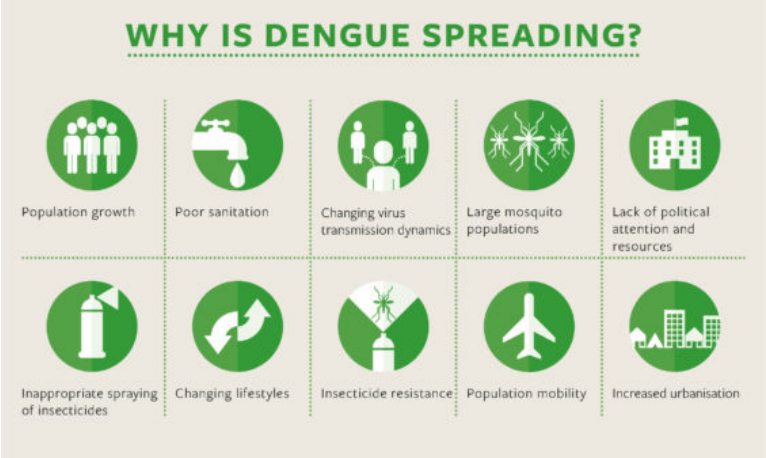

Image courtesy of Malaria Consortium

The researchers also explained that they didn't want people to kill the larvae, just to introduce this fish. "We told them that the fish are part of

the normal eco-system and this is how the fish need to survive," says John. "It's part of the normal circle of life."

SO FAR, SO GOOD

Throughout the study, which ran from October 2015 to September 2016, the number of adult Aedes females per household was significantly higher in the group using traditional methods. The study, which won the 2017 Break Dengue Community Action Prize, also found that people accepted guppies well and liked the SumiLarv® 2 MR devices, though the community needed to understand how PPF works and the role of the adult mosquito in the transmission of dengue in Cambodia. Furthermore, a tailored engagement approach and communication materials using COMBI led to high levels of community acceptance and participation.

On the question of sustainability, while it is probably too early to say if it will be sustainable in the long run, the guppy fish are still there. "We were able to follow-up thanks to prize money from Break Dengue. We met with the guppy bank and the health center folks using the guppy bank. People are still enthusiastic about it, but we need to see how it progresses with time," concludes John.

How is your community taking the fight to dengue? We want to hear your stories.

—

Click below to report dengue activity near you and get access to up-to-the-minute, crowdsourced reports on outbreaks.

Dengue Track

Appendix 6.8 Community action prize end of project report

Break Dengue Community Action Prize End of Project Report

Submission date: November 25, 2017
Submitted to: Nicholas Brooke, The Synergist

Submitted by: John Hustedt
House #91, Street 95,
Boeung Trabek, Chamkar Morn, Phnom Penh
Cambodia
Email: johnhustedt@gmail.com
Telephone: 855-898-63204

Acronyms

CHWs	Community Health Workers
COMBI	Communication for Behavioral Impact
FGDs	Focus Group Discussions
IDIs	In-depth Interviews
IVM	Integrated Vector Management
HC	Health Center
NDCP	National Dengue Control Program
NGOs	Non-government Organizations
WHO	World Health Organization

Introduction

The Break Dengue team inform us that our initiative "Implementing integrated vector management for dengue control", was chosen as the winning submission of the Break Dengue Community Action Prize on Feb 21, 2017. As much of our original idea had already been accomplished by that time, the project manager (Aaron) worked with us to develop some goals listed below:

National Strategic Plan Development

- Discussions for Development of Plan (April- May 2017)
- Finalization of Plan (June-July 2017)
- Promotion of Plan/Advocacy to Donors (July-September)

Community Health Worker - Integrated Vector Management Project

- Follow-up Survey including questions on vaccine barriers and community thoughts (May-June 2017)
- Development of integrated VC/Vaccine health education materials (June-September)

Activities

National Strategic Plan Development

The Dengue Integrated Vector Management (IVM) team participated in initial discussions with National Dengue Control Program (NDCP), World Health Organization (WHO), and other partners on the development of a National Dengue Strategic Plan. This included attending a stakeholder meeting on the proposed national plan on March 28, 2017 (See Annex 1). John attended a separate side meeting with Dr. Hasitha Korelege, WHO consultant, who was leading the development of the plan on the evening of March 28. During the meeting John shared his experience working with NDCP staff, gaps in the strategy, possible ways forward, and items to ensure make it into the plan. The introduction of a combination of vector control and vaccines was discussed. The plan has not as yet been finalized by the working group as planned, but our team has continued to give input and support to the NDCP where needed.

Community Health Worker - IVM Project

As proposed, a follow-up survey was completed on 07-08 August 2017. The team included two supervisors (from the IVM team), two experienced moderators, and two experienced note takers. An initial training for moderators and note takers was held the week before. Data collection included four Focus Group Discussions (FGDs) and two In-depth Interviews (IDIs) as described below:

- 2 FGDs with Community Health Workers (CHWs) (8 people each)
- 2 FGDs with Community Members (8 people each)
- 2 IDIs with Health Center (HC) Chief (2 people)

The protocol for FGDs and IDIs followed that of the previous project which can be found in our published protocol (https://trialsjournal.biomedcentral.com/articles/10.1186/s13063-017-2105-2). The topic guides and script (in short form) can be found in Annex 2 and 3, respectively. Following the events villagers were asked to draw dengue prevention methods including the use

of vaccines. These drawings were used to add a section on vaccination in the Community Health Worker flip chart.

Results

FGDs with Community Health Workers

CHWs suggest there is an increased understanding about dengue prevention, and improved health seeking behavior. CHWs believed that dengue caused a reduction in mosquito numbers and dengue incidence. When asked to list and rank all potential vector control options every participant preferred guppies. The reasons given include that they make the water clean, easy to look after, involve no financial costs, and the fish are beautiful. The option the majority liked the least was bringing smoke.

The fish are very useful because guppy fish eat the larvae. The villagers clean and cut the forest around the house. Interestingly there have been no dengue cases in the village and when they have fever, they go to the hospital immediately. The villagers know mosquito bites cause dengue.

"Now I do not bring the guppy fish to the villager house because they come to get the fish by themselves"

However, a year after the conclusion of the Integrated Vector Management project there is relatively less focus on management of the breeding jars and less fish in villager's homes. This is due to competing priorities with CHWs time, and less follow up at HC monthly meetings. Villagers also experienced difficulties with fish disappearing during rainy season, and frogs and geckos eating the guppy fish. Challenges in maintaining the guppies included not having support for travel to monthly meetings at the health center they sometimes were not able to take needed fish from guppy banks. The main disadvantages of guppies were that in some cases they had many children leading to large numbers of guppies in some jars, and some individuals (especially those that migrate for work) did not replace them after they died.

"Other animals eat them such as frogs, the fish eat each other, and if there are no fish in the jar they are lazy to come to get the fish to fill in sometimes"

Most of the CHWs had not heard of the dengue vaccine although a few had heard of it through Facebook. Most are positive and were interested, although they want to know more about the efficacy and safety of the vaccine. Some CHWs also suggested they would feel more comfortable if the national program rolled it out for the whole country. However, the government should be careful about misconceptions and what communication messages are provided as many CHWs felt that one vaccination would prevent them from getting dengue for the rest of their life. It will also be important to install the message that using imperfect vaccines means they still need to carry on with environmental management, vector control, and continue encouraging proper health seeking behavior. They were willing to pay between $5-10 for the vaccine, however, would prefer the vaccine be provided free by the government. When asked who should be responsible for the vaccine, they all suggested the public sector through the Ministry of Health and Non-government Organizations (NGOs) rather than the private sector as is currently the case in Cambodia.

"Only one time getting vaccinated can prevent dengue your whole life and you can stop worrying about dengue."

"Even the dengue vaccine is available we still do prevention because it is does not prevent 100% and we can prevent transmission to the other people who don't have the vaccine."

"Even the dengue vaccine is available we will still use the guppy fish, provide health education, and clean the house. It can help to prevent from other diseases transmit by mosquitoes."

FGD with Community Members

Community members had a good understanding of dengue signs and symptoms and showed appropriate serious attitudes towards the disease. They were able to identify that dengue came from *Aedes* mosquitoes and identify the peak season of transmission. They understood prevention methods (including environmental management and methods to prevent biting). Vector control methods were ranked with Guppies as the most preferred followed by environmental management and sleeping under nets during the day. Mosquito sprays and repellents were disliked by all repellents due to fears of health effects and high costs.

"The guppies were given by village chiefs and neighbors. We like the guppy fish because the guppy fish eat all the larvae in the jars and keep the jar clean."

"People dislike chemical insecticide; it does effect health and it costs a lot."

Health seeking behaviors were well understood with communities reporting that after suspecting dengue all individuals should be brought to the clinic for evaluation, however they reported that

most people did wait 1-2 days before bringing their child to care at the hospital. They reported the main barriers to treatment as issues related to transportation and payment (e.g. long travel distances to health centers, clinics closed during night times, and high costs of private clinics).

"Mostly 2-3days after fever will go to the hospital. Parents play the most important role to decide to go to the hospital."

Community members felt that the most important achievements of the project were to provide health education and start up the guppy fish distribution system. They also mention that there has not been dengue in their village, and they attribute this to the interventions. This is important to note and although encouraging that there is a reduction in perceived cases the true incidence is unknown. Additionally, even if there is a true reduction without elimination then additional cases in the future may make the community feel the interventions are no longer working. This highlights the importance of providing true information and realistic expectations among communities in the intervention.

"Using the guppy fish results in no mosquitoes and no dengue because guppy fish eat all the larvae."

The community found that the greatest challenges to maintaining the guppies was that they are eaten by other animals such as lizards and frogs and disappearance when water jars overfill. Communities would be willing to pay around $0.10 for a guppy.

None of the community members had heard about the dengue vaccine. Some community members noted that they preferred guppy fish or larvicides because they reduced mosquito bites (including nuisance biting). However, other community members would prefer a vaccine as guppy fish/larvicides can only be put in one place, while a vaccine could protect you wherever you go. Community members all suggested they would be willing to pay between $1.25-6.25 per dose, except for one person who was willing to pay up to $25. However, they all said they would take it should the government be willing to provide it for free. When asked who should be responsible for the vaccine, they all suggested the public sector through ministry of health and NGOs rather than the private sector as is currently the case in Cambodia.

"Even if the vaccine is available, we need to do the interventions such as cleaning the house and distributing the guppy fish, because it can prevent the other people who don't get the vaccine."

"If the dengue vaccine becomes available that will be great because it can prevent us from dengue, and we can stop worrying anymore and don't have to spend the money on treatment."

"Only one person is willing to pay 50000-100000 riels ($12.5-25) because they say a one-time injection can prevent dengue for their whole life."

IDI with Health Center Staff

Health center staff mentioned that they still have monthly CHW meetings and distribute guppy fish to CHWs from the HC bank. They both still viewed the cultivation and distribution of guppies as part of their responsibility at the HC after the project ended. Both feel the interventions resulted in decrease in dengue cases even after the project ended (although that is their opinion and the real incidence is unknown). They both said that villagers preferred guppies over other alternatives because the fish eat the larvae immediately, leave the jars very clean, relatively easy to take care of, and easily produce new babies. They both felt that villagers were continuing to use guppies, and that some even came to the health center themselves to take the fish. They felt the most preferred Communication for Behavioral Impact (COMBI) activity was the Tuk Tuk advertisements.

"The people still use the fish and they come to take guppy fish from health center. The health center continues to advertise to the people visiting the health center every day."

"Before when people are sick they pray first before bring to the hospital but now they are understand and change the behavior when sick go to hospital to check."

"There are five villages using guppies, they are watched after very well and have reduced the cases of dengue to nearly none."

"Using the guppy fish is preventing dengue. The villagers like the guppy fish because they are colorful, eat the larvae immediately, and leave no larvae in the container."

A year later, one said that he continues to have monthly meetings with the CHWs and encourages them to continue health education and provide guppies. They also requested that the guppy fish work be extended into other villages were not included in the pilot. Although the number of individuals using guppies in project villages has decreased some, there has been an overall increase with several other villages previously not involved that have begun raising the fish. The health center staff feel this is because the COMBI messages helped them understand and accept the interventions.

"Guppy fish are easy to take care, can eat the larvae immediately, and can produce a lot of new generations."

"Before when people are sick they pray first before bring to the hospital but now they are understand and change the behavior when sick go to hospital to check."

Both HC staff had heard of the new vaccine from media and colleagues and thought that the community would be very accepting of it. However, there is still some misunderstanding of the vaccine in general among one of the HC with them saying that the vaccine could prevent people their whole lives. The other mentioned that vaccines are not always able to prevent 100% of infections and they would continue with prevention activities and support positive health seeking behavior. They felt that the community would be willing to pay $1.5-5 for the vaccine, and that they would definitely take it if it was free. They both felt the responsibility for vaccine should be the responsibility of the government rather than the private sector.

"If the vaccine available does not provide 100% prevention, we need to put the guppy fish for additional prevention."

Impact and Achievements

As the results of our IVM project are just starting to come out in peer-reviewed journals and being prepared for additional scientific conferences, the dengue prize funds were helpful in considering how sustainable such interventions may be in a community. The above FGDs and IDIs suggest that there is still demand in many villages for the interventions, and that even some villages which part of the project were not are now adapting them in their communities with support of the HCs. This shows that demand creation in villages over a relatively short period of time can lead to medium term acceptance in some cases.

This work helps highlight some of the successes and challenges experienced in the communities after the end of the project. Important successes include the continued efforts by HC staff and CHWs to breed guppies and keep the system functioning. The sustained interest by the community in guppies and improved health seeking behaviors are great achievements of the COMBI activities. However, the work also highlighted continued challenges including the loss of guppies to frogs, lizards, and other animals, the reduced motivation in some CHWs after the end of the project, and the reduction in HC meetings in some areas. These are all important issues to note and need to be considered when designing any future strategies for larger scale implementation.

The dengue prize money also helped unearth health workers and community member's feelings and attitudes about the dengue vaccine. The results show that most people are overwhelmingly in support of implementing a dengue vaccine, however some issues remain to be considered for COMBI activities should a vaccine be introduced. Most importantly these include the continued emphasis on environmental management in the house and efforts to reduce mosquito biting as this can reduce transmission of other vector borne diseases as well. It will also be important to ensure messages around proper health seeking behavior still become part of the package, and that the true efficacy of any vaccine is properly communicated (e.g. taking the vaccine may not mean lifelong immunity to all four serotypes and you should not abandon mosquito and disease prevention efforts).

Additionally, support from the IVM team has helped inform and impact the national dengue control policy. This is evident by WHO staff insisting to include guppy introduction into the plan as one potential vector control method. The WHO staff in charge of climate change adaptations have also included a request for funds for guppy introduction into the Cambodia Climate Change Alliance proposal, and another internal proposal they are writing. Discussions about the introduction of the current or future dengue vaccines were also facilitated with both the WHO vector-borne disease unit and the expanded program for immunization. Continued discussion between the government and WHO on vaccine introduction will be important to create the right policy for Cambodia.

Overall, the dengue prize money has been able to facilitate continued discussions around dengue control policies and has directly impacted the creation of such policy and funding related requests. Hopefully these activities will lead to better dengue control in Cambodia and better health for the all the Cambodian people.

Appendix 6.9: Rural Cambodia uses guppy fish to fight dengue

REUTERS

ENVIRONMENT
DECEMBER 1, 2016 / 11:25 PM / 3 YEARS AGO

Rural Cambodia uses guppy fish to fight dengue

Astrid Zweynert

LONDON (Thomson Reuters Foundation) - In the backyards of rural Cambodia, a tiny weapon is being deployed to fight dengue fever, the world's fastest spreading tropical disease that causes debilitating flu-like symptoms and can develop into a deadly hemorrhagic fever.

More than 3,000 households in Kampong Cham province, which has one of the highest dengue rates in Cambodia, have been given colorful guppy fish to breed in barrels of water that villagers keep close to their homes for cleaning and cooking.

Presenting the results of a one-year pilot project, charity Malaria Consortium said the larvae-eating guppies have helped to reduce the presence of potentially dengue-carrying mosquitoes by 46 percent during the trial at a cost of a few cents per fish.

"The idea was to create a sustainable solution for the villagers, with minimal costs and inconvenience for them," John Hustedt, senior technical officer at Malaria Consortium Cambodia, told the Thomson Reuters Foundation.

The project is part of a growing trend to find cheap, low-tech solutions to medical problems, especially in developing countries where disease outbreaks can severely test already stretched health budgets, experts said.

The Malaria Consortium hopes the Cambodian government will agree to roll out its guppy project across Cambodia, which reported almost 200,000 dengue cases between 1980 and 2008, one of the highest rates in Southeast Asia.

FALLING THROUGH THE CRACKS

Dengue has spread to more than 100 countries from nine in 1960, according to the World Health Organization, and explosive outbreaks have become more common.

Almost half of the world's population is at risk from dengue with cases rising to 390 million a year from 15,000 in 1960.

Experts say the increased movement of people and goods due to globalisation as well as a rise in floods linked to climate change are likely to speed up the spread of dengue.

The economic cost is potentially huge, with the disease estimated to cost the Americas $2.1 billion annually, while Southeast Asian economies could lose almost $2.4 billion.

Yet global attention and funding to fight the disease have been limited, not least because mortality rates are much lower than from malaria and three quarters of those infected do not show signs, making it a "silent disease".

"The approach to dengue is reactive - when there is an outbreak. We have to get more pro-active in controlling it before it becomes an even greater threat," said James Tibenderana, Malalaria Consortium's global technical director.

There is no dedicated treatment for the virus. Patients are generally asked to rest, drink plenty of fluids and take medication to bring down fever and reduce joint pains.

Simple tools such as testing blood from a finger prick to diagnose malaria are not available for dengue, and there is no routine testing for the disease if a malaria test is negative.

Clinical trials of a new vaccine have been promising, a recent study showed, but despite 70 years of effort, a vaccine with high efficacy remains elusive.

Appendix 6.10: Guppy fish making a splash in dengue fever prevention

Guppy Fish Making a Splash in Dengue Fever Prevention

By **Hannah Hawkins** - December 3, 2016

Guppy fish—a colorful species just 3 to 4 centimeters in length—are being heralded as a potential way of preventing dengue fever outbreaks in Cambodia.

The Malaria Consortium just wrapped up a yearlong pilot program in Kompong Cham province that set out to find the most cost-effective way of preventing outbreaks in a country that has one of the highest per-capita dengue incidence rates in Southeast Asia.

The project zeroed in on the guppy, a tropical fish—referred to as the "seven-colored fish" by Cambodians—that feeds on the larvae and adult Aedes aegypti mosquito, which carries the virus, and thrives in stagnant water.

In the trial, guppies were added to household containers holding at least 50 liters of water, such as drums and water jars. When the water was tested throughout the trial, those households with fish had less than half the adult mosquitoes of those without.

With no vaccine or widespread treatment currently available in Cambodia, prevention that relies on controlling mosquito populations is the best alternative, according to Rabindra Abeyasinghe, a World Health Organization specialist. "Even if we have vaccines, we still need vector control," he said on Thursday. "Aedes flourishes better in our cities than we do."

Mr. Abeyasinghe, whose organization endorses the Malaria Consortium's project, said guppy fish are the best solution for Cambodia at the moment.

The "guppy fish doesn't pollute the environment—we are happy to be a part of that," he said, adding that the scale and frequency of dengue outbreaks will fall under the plan, reducing the dependency on pesticides.

There were 14,303 reported cases of dengue in Cambodia last year, and 35 deaths, National Dengue Control Unit program manager Rithea Leang said at a news conference organized by the Malaria Consortium in Phnom Penh on Thursday.

According to the consortium, an international NGO that focuses on the control of communicable diseases, about 13 percent of the cases were found in Kompong Cham province, the location of its trial.

Mr. Leang said the current national strategy was to treat water in dengue-prone areas with larvicide once a year. However, John Hustedt, the project's senior technical officer, said this is only done once

an outbreak—which is defined by the government as three or more cases in one village—is identified.

Despite acknowledging that the government's current plan is more costly than using guppy fish, Mr. Leang said his department, which he repeatedly stressed did not have a big budget, was "not convinced" that the fish were a long-term alternative.

"We don't know how sustainable it is yet—it is too early," he said, adding that they were concerned about the plan's practicality and manageability.

Marian Blondeel, a spokeswoman for the consortium, said the government still "believes there is some value in conventional methods," including the use of larvicides and insecticides. "They do see the benefits [of guppy fish], but change is always hard."

Although Mr. Leang seemed hesitant about the new strategy, Yves Bourny, country director for the Malaria Consortium, said it was always his organization's intent to raise the funds for a national roll-out.

It would cost about $1 million to expand the plan to 2 million people across five provinces that are most prone to dengue outbreaks, but donors are interested in supporting a plan that is more effective and sustainable than insecticides, he said.

"There is evidence that there could be resistance to insecticides," Mr. Bourny said. "The era of insecticides is over."

Ms. Blondeel said community members involved in the pilot program were big fans of the fish—and not just because of the hard data.

"We asked the communities what they thought of [the project], and they all said they really like the fish—they see the fish as an omen of good luck in Buddhism," she said.

Kim Sourphirum, director of Kompong Cham's provincial health department, said dengue cases in the province had gone down from last year—1,031 cases were reported this year, compared to 1,556 last year—but he could not confirm a direct link to the guppy project.

"It's a good project because the guppy fish can kill mosquitoes before they are born," he said. "I hope the plan keeps going."

Thai Sokheng, a 53-year-old from Kompong Siem district's Choeung Kuok village, which was one of the areas treated with guppy fish, said villagers did not trust the conventional dengue control method.

"They used to complain and worry about larvicide," Ms. Sokheng said. "In my area, many villagers support having guppy fish."

"I hope this project continues forever," she said.